ULTRASONOGRAPHIC EVALUATION OF ORAL SUBMUCOUS FIBROSIS TREATED WITH ORAL PENTOXIFYLLINE AND INTRALESIONAL INJECTION OF DEXAMETHASONE WITH HYALURONIDASE

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CERTIFICATE

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I **SURESH KUMAR M,** hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Tamil Nadu Government Dental College and Hospital, Chennai-600003. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in the dissertation. The author has the right to reserve for publish of work solely with the prior permission of the Principal and Guide, Tamil Nadu Government Dental College & Hospital, Chennai-600003.

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CONTENTS

S.NO	TITLE	PAGE NO
1	INTRODUCTION	1
2	AIM AND OBJECTIVE	4
3	REVIEW OF LITERATURE	5
4	PATHOGENESIS OF OSMF	17
5	PHARMACOLOGY OF PENTOXIFYLLINE	26
6	PRINCIPLE OF ULTRASONOGRAPHY	33
7	MATERIALS AND METHODS	39
8	STATISTICAL ANALYSIS	47
8	RESULTS AND OBSERVATION	53
9	DISCUSSION	56
10	SUMMARY AND CONCLUSION	62
11	BIBLIOGRAPHY	64
12	APPENDIX	

LIST OF ABBREVIATIONS USED

BQ	Betel quid
cAMP	Cyclic Adenosine monophosphate
COX-2	Cyclo-oxygenase 2
ECM	Extracellular Matrix
HLA	Human Leukocyte Antigen
HO-I	Heme oxygenase-I
IL 1	Interleukin 1
IL-6	Interleukin 6
IL-8	Interleukin 8
INF-γ	Interferon Gamma
LOX	Lysyl Oxidase
MMP3	Matrix metlloproteinase 3
OSMF	Oral Submucous Fibrosis
PAI	Plasminogen Activator Inhibitor
TGF-β	Transforming Growth Factor - Beta
TIMPs	Tissue Inhibitor of Matrix Metalloproteinases
TNF	Tumor Necrosis Factor
USG	Ultrasonography
VAS	Visual Analogue Scale

INTRODUCTION

Health is multifactorial and multidimensional, influenced by various factors. Disease is generally an individual problem and infirmity is probably a result of genetic makeup and environmental influences. Several disorders that affect the oral mucosal health are acquired through various lifestyle practices. Addictions for any adverse habit merely represent man's unbounded weakness. Such adverse habits may follow the ladders of culture or mere addiction; which forms a platform for disease to show its synchronous presence. In day to day clinical practice Dentists often encounter a wide spectrum of oral mucosal alterations. These mucosal alterations may be in the form of changes in the colour, size, shape and texture. They range from innocuous mucosal alterations needing simple therapeutic remedies to more interventional procedures. Many of these lesions are caused by habit of betel nut and tobacco use. Oral Submucous Fibrosis (OSMF) is one among them which is frequently encountered due to betel nut chewing.

Schwartz in 1952 described a condition affecting the oral mucosa including the palate and faucial pillar, called "atrophia idiopathica (tropica) mucosae oris" among five Indian women from Kenya[1]. Later the term "Oral submucous fibrosis" was coined by **S.G Joshi in 1953**[2]. It was also called by the other names like "idiopathic scleroderma of the mouth", "idiopathic palatal fibrosis", "juxta-epithelial fibrosis". **Paymaster** (1956) observed the precancerous nature of OSMF, because of the slow onset of squamous cell carcinoma in one third of OSMF patients[3].

In 1966 **Pindborg and Sirsat**, defined OSMF as "an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always

associated with juxta-epithelial inflammatory reaction followed by fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat"[4].

The disease is predominantly seen in India among Asian countries, with a reported prevalence ranging up to 0.4% in Indian rural population. Based on study conducted in 2002, more than 5 million people in India suffer from OSMF (0.5 % of Indian population). OSMF is widely prevalent in all age groups and across all socioeconomic strata in India[5].

Several factors play a role in the etiopathogenesis of OSMF and current evidence suggests that arecoline in the areca nut is the key factor in initiating the disease[6]. The habit of betel quid chewing is widespread throughout India and South East Asia. And it is widely prevalent in teenagers and young adults[7].

Buccal mucosa, faucial pillar, soft palate are predominantly affected. Underlying muscles and the muscles of mastication can also be involved. The mucosa in the involved areas gradually becomes pale followed by progressive stiffness of subepithelial tissues. Increased incidence of malignancy is noted in OSMF patients which is around 8% in overall affected Indian population[8].

Many treatment protocols have been proposed for OSMF to alleviate the signs and symptoms which include intralesional steroids, hyaluronidase, placentrax, immunomodulatory drugs, immunised cow milk[9], colchicine[10], antioxidant, nutritional supplements, physiotherapy, combined medical therapy and surgical therapy with varying degrees of benefits[11]. But none of them proved to be effective due to their own short comings.

Currently pentoxifylline, tri-substituted methylxanthine derivative, is reported to have satisfactory result in the management of OSMF due to their immune

modulation, alteration of fibroblast physiology, rheologic modification and anti-inflammatory property. Immunologic abnormalities have been primarily reported with OSMF which probably mediate local tissue damage and that they appear to be the final common pathway in the pathogenesis of OSMF. Pentoxifylline has immunomodulating effects which include increasing leucocyte adhesion, causing neutrophil degranulation and release of peroxides and decreasing production of tumour necrosis factor alpha[12]. The review of current literature shows that the use of pentoxifylline in OSMF management has not been adequately explored and research in this area is very limited. Very few studies have been done so far, that too mainly involve subjective evaluation like mouth opening, blanching of oral mucosa, burning sensation. Objective evaluation of treatment outcome in OSMF patient can be carried out using ultrasonography.

Ultrasonographic(USG) imaging is considered as a "real-time" imaging, which generate electrical impulses that are converted into high frequency sound waves by a transducer and transmitted into the tissues being examined. It is then reflected as echoes and reconverted into electrical energy, amplified, processed, and displayed on the monitor[13]. USG is particularly suitable for imaging superficial structures of the oral mucosa and provides both quantitative and qualitative assessment, the nature and dimension of lesion or structure[14].

The purpose of the study is to evaluate objectively the effectiveness of oral pentoxifylline for the treatment of OSMF using high frequency ultrasonography in comparision to conventiontal intralesional steroid therapy with dexamethasone and hyaluronidase.

AIM AND OBJECTIVE

AIM:

To evaluate the therapeutic efficacy of Oral Pentoxifylline in treatment of oral submucous fibrosis patients using ultrasonography.

OBJECTIVE:

1. To assess the clinical symptoms like burning sensation, mouth Opening, both preoperatively and postoperatively.
2. To measure the Sub mucosal layer thickness in oral submucous fibrosis patients, pre and post treatment using ultrasonography
3. To assess the Echogenecity of buccal mucosa in oral submucous fibrosis patients, pre and post treatment using ultrasonography

REVIEW OF LITERATURE

HISTORY

OSMF has been well established in Indian medical literature since the time of **Susrutha,** a renowned Indian physician, who described a condition resembling OSMF as *Vidari* under mouth and throat during the period of 2500-3000 BC. He noted pain on taking spicy food, depigmentation of oral mucosa and progressive narrowing of oral cavity[4].

Schwartz (1952)[1] for the first time reported a case of "Atrophia Idiopathica tropica mucosae oris" occurring in Indians in East Africa and described blanching and stiffness of the oral mucosa, difficulty in opening the mouth and inability to tolerate spicy food.

Sirsat (1962)[15] an ENT surgeon from India first described this condition and coined the term "submucous fibrosis of palate and pillar of fauces". **Rao (1962)**[16] observed the fibrous bands extending to the lateral wall of pharynx via the faucial pillars down to the pyriform fossa.

Pindborg and sirsat (1964)[17] coined the term *Oral Submucous fibrosis*. Pindborg JJ et al (1965) studied the frequency of oral submucous fibrosis among 10 south Indians with oral cancer. Forty of the 10 oral cancer patients had clinical signs of submucous fibrosis.

Mathew B et al (1967)[18] reported oesophageal involvement in 5 of 11 OSMF patients.

EPIDEMIOLOGY:

Pindborg JJ et al (1968)[5] in their epidemiological survey concluded that there is an increased incidence of OSMF of about 0.18%-1.2% in urban population when compared with 0.04% - 0.4% in rural population.

Ranganathan K et al (2004)[19] conducted a study in Chennai, South India, reported a mean age of 32.4±10.4 years and median age of 29 years. The youngest and oldest ages of occurrence of OSMF in this study was 16 and 76 years in males and 24 and 57 years in females. Occurrence of the disease in individuals as young as 2, 3.5, 4, 11 and 12 years has also been reported.

ETIOLOGY:

Wahi PN et al (1966)[20] in their study reported OSMF to be higher in patients with poor nutritional status. They found that the patients with OSMF showed a higher frequency of deficiency of vitamin A, B, C and multiple vitamin.

Gupta PC et al (1966)[21] stated that oral use of any tobacco product like gutkha contain arecanut and several other substances in powdered or granulated form which causes oral submucous fibrosis

Phatak AG (1978)[22] did a study on 34 patients suffering from OSMF and observed that these patients had significantly elevated levels of serum globulin and immunoglobulin IgG, stating the possibility of OSMF being an auto-immune disorder.

Canniff JP et al (1986)[11] suggested that the addition of slaked lime to areca nut hydrolyses arecoline to arecaidine which means that the hydrolysis of arecoline could occur in the saliva as well as in fibroblasts. It was also suggested that the inflamed mucosa had enhanced permeability to arecoline and arecaidine.

Van wyk CW et al (1988)[23] have reported about irreversibility nature of the disease i.e., once OSMF induced by the habit of chewing betel nut, the reversal of the disease after cessation of the habit could not occur.

Sinor PN et al (1990)[24] demonstrated the Dose-response relationship – the relationship between degree of exposure and the risk of a disease is an important

criterion for causal inference. In a case-control study from India, the relative risk increased with the duration as well as the frequency of the areca nut chewing habit. In a bivariate analysis of the duration and frequency of the habit, the risk of OSMF showed a clear dose-response relationship

Jeng JH (1994)[25] conducted a study to observe the pathobiological effects of aqueous extracts of three betel quid constituents, inflorescence of piper betel, arecoline and catechin on cultured oral mucosal fibroblasts. Result showed that betel quid contained not only genotoxic and cytotoxic agents but also compounds that had the ability to stimulate cellular proliferation

Murti PR et al (1995)[7] found on their tissue culture experiments using human fibroblasts and suggested that arecanut alkaloids yield powerful carcinogenic nitrosamines which explains the malignant potential of OSMF.

Trivedy C et al (1999)[26] in their study observed that copper is released from areca products during chewing and is deposited in oral tissues. They found that lysyloxidase activity is upregulated in OSMF patients.From these findings they hypothesized that cellular events lead to cross linking of collagen and elastin, making them less degradable. The upregulation of lysyloxidase in OSMF may be an important factor in the pathogenesis of this disorder.

Chen HM (2004)[27] conducted study on HLA typing in Taiwanese patients and found significantly greater phenotype frequency of HLA-B76 and haplotype frequencies of HLA-B48/Cw7, B51/Cw7 and B62/Cw7 in OSMF patients than in healthy control subjects. These findings suggested that some Taiwanese areca quid chewers with specific HLA phenotypes or haplotypes are prone to have OSMF

Tu HF et al (2006)[28] conducted on the functional polymorphisms of matrix metalloproteinase 3 gene among male OSMF patients using areca, reported that the

5A genotype in MMP3 promoter was observed more frequently in OSMF patients than in controls. The results indicated that the 5A genotype of MMP3 promoter was associated with the risk of OSF

CLINICAL SYMPTOMS

Rao AB (1962)[16] noted features like inability to open mouth and intolerance to hot spicy food in 35 of 46 patients in his study, 3of 46 patients could not blow out a candle and had inability to protrude their tongue, 15 of 46 patients complained of pain in the ear and 6 of 46 patients complained of swelling and pain around the lower jaw and neck

Pindborg JJ and Sirsat (1966)[4] in their article noted prodromal symptoms like burning sensation for spicy food, blisters and ulceration or recurrent stomatitis, defective gustatory sensation, excessive salivation and dryness of mouth on OSMF patients.

Phookan J et al (1998)[29] studied 32 patients with different oral lesions over the time span of one year and found incidence of OSMF to be 0.2%. Buccal mucosa was the common site of involvement, and white discoloration of mucosa was the common presenting symptom. All the cases in the study were found to be associated with betel nut chewing habit.

Sumeth Perera MWD et al (2007)[30] in their studies on the areca nut-treated oral epithelium showed progressive changes in epithelial thickness leading to epithelial atrophy, fibrosis of connective tissue with increased cellularity, focal inflammatory cells infiltrate and muscle atrophy. This study concluded that evidence of areca nut contribution to the development of OSMF in treated animals has been elucidated.

Reichart et al (1984)[31] studied 15 biopsies of oral mucosa of betel chewers who were studied histologically and by transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Histologically marked reduction of retepegs, epithelial atrophy, hyper ortho or parakeratosis, sub-epithelial edema and inflammatory infiltrate were the prominent findings

El Labbon et al (1985)[32] have investigated the muscle changes ultrastructurally in OSMF patients and concluded that restricted mouth opening in OSMF might be depend upon not only on subepithelial fibrosis, but also on the extent of degeneration of masticatory muscle.

Reichart PA et al (1994)[31] concluded in their study that the process of fibrosis starts in the deeper subepithelial connective tissue stroma and not close to the subepithelial basement membrane.

Cox SC and Walker DM (1996)[33] in their review article stated that the retromolar areas and the buccal mucosa were commonly involved, followed by soft palate, palatal fauces, uvula, tongue and labial mucosa. The main diagnostic criteria for OSMF clinically was the presence of fibrous bands either in the buccal or labial mucosa and in the posterior part of palate.

Tilakarathne WM et al (2006)[34] reviewed about the disease progression and sequential disabilities of oral functions like restricted mouth opening, inability to blow out a candle or whistle and difficulty in swallowing. They concluded that bands are common at the back of the mouth in mild cases of OSMF and as the disease increases in severity are more likely to be found anteriorly as well.

PRECANCEROUS POTENTIAL:

Paymaster JC (1956)[3] was the first to mention submucous fibrosis as a premalignant condition. He reported slow growing of squamous cell carcinoma in 1/3rdof his patients with OSMF.

Pindborg JJ (1972)[35] summarized a criteria to support the precancerous nature of this disease as

- Higher prevalence of leukoplakia among OSMF patients.
- Higher frequency of epithelial dysplasia.
- Concurrent findings of OSMF in oral cancer patients.
- Histopathological diagnosis of oral cancer without clinical suspicion among OSMF cases.
- Higher rate of incidence of oral cancer among patients with OSMF

Murti PR et al (1985)[8] proposed that the malignant transformation rate has been reported to be 4.5% over a 15 year period. With the further follow up for another two years showed that the rate of malignant transformation arose to 7.6%. Oral cancer developed 3-16 years after the diagnosis of submucous fibrosis. The average age at the time of malignant transformation was 64.6 years and age range was 48-81 years.

Dave et al (1991)[36] reported a statistically significant increase in the size C-band heteromorphism patterns on chromosome 1 in submucous fibrosis and oral squamous cell carcinoma patients when compared with healthy subjects. In another cytogenetic study by the same author, an increase in the frequency of sister-chromatid exchanges and chromosome aberrations in areca nut consumers with OSMF and OSCC was noted.

TREATMENT MODALITIES:

Sinha and Jain (1978)[37] have tried multiple submucosal injection of 1.5 cc hydrocortisone in OSMF patients and found to be effective in controlling symptoms and alleviating inflammatory findings.

Kakar PK et al (1985)[38] observed in a group of 96 patient who received 4 regimens of treatment which were intralesional dexamethasone or hyaluronidase, combination of dexamethasone and hyaluronidase and local placental extract. The group of patients receiving hyaluronidase alone showed faster improvements in symptoms. However combination with dexamethasone gave better longer term results.

Gupta et al (1988)[39] reported successful treatment of OSMF with local injection of chymotrypsin, hyaluronidase and dexamethasone.

Borle et al (1991)[40] concluded from his studies that all available treatment for OSMF are basically palliative in nature and provide only symptomatic relief and retard the manifestations of the disease. They also suggested that conservative treatment is safer and less hazardous than treatment with submucosal injections.

Dinesh CG et al (1992)[41] suggested that no one treatment modality is successful in completely eliminating the disease. In patient with grade III and grade IV injectable corticosteroids or hyaluronidase locally is helpful and to prevent relapse placentrex and ranindone combination is advised.

PENTOXIFYLLINE:

Occlusive blood vessels encountered in OSMF restrict nutrients and therapeutic substances from reaching the affected tissue, which may be one of the reasons for the unsatisfactory therapeutic effect of local medical treatment. Thus

drug with vasoaltering property were started using in the management of OSMF.

Lai et al (1995)[42] has tried management of OSMF using buflomedial HCL (3 tablets of 45mg per day) and topical trimacenolone acetonide 0.1% ointment on mucosal ulcers at bed time. He observed better results. Buflomedial HCL (peripheral vasodialator) has been found to improve the the tissues with diffuse fibrosis to a noticeable degree by the relief of local ischemic effect.

Recently Pentoxifylline, a methylxanthine derivative has been found to exert fibrinolytic effect on cultured fibroblasts or animal models of fibrosis, including liver fibrosis. In hepatic stellate cell culture, Pentoxifylline has been shown to inhibit liver fibrogenesis.

Rajendran et al (2006)[43] used pentoxifylline which has rheologic modifying properties. It was administered as 400mg thrice daily for a period of more than 12 months and observed improvement in symptoms of OSMF.

Mehrotra et al (2011)[44] studied the effectiveness of pentoxifylline in 29 cases of OSMF for a period of 7 months and concluded that it has found to be effective in alleviating the symptoms and can be used as adjuvant in the management of OSMF.

Anjum Aara et al (2012)[45] studied clinically to evaluate the efficacy of oral Pentoxifylline 400mg in OSMF patients in comparison to intralesional injections of Dexamethasone (4mg/ml) and Hyaluronidase 1500 IU in the management of OSMF patients. He concluded that Pentoxifylline can be safer and better alternative in the treatment of oral submucous fibrosis in comparision to dexamethasone.

Various method have been employed to evaluate the outcome of medical management of OSMF which were mainly subjective, like alleviation of symptoms like burning sensation, alteration in texture of mucosa, changes in blanching of

mucosa and improvement in mouth opening. Ultrasonography proved to be effective in evaluating the progression and treatment outcome in course of time. It is non invasive, real time imaging modality helpful in identifying the distribution of fibrosis and submucosal changes with medicinal therapy.

ULTRASONOGRAPHIC EVALUATION:

Professor **Ian Donald**[46] of Glasgow is credited with being the first to successfully use diagnostic ultrasonography to investigate the gravid uterus and is considered as the father of modern ultrasonography.

The echogenecity of a tissue primarily relates to its stiffness, the chief source of which is collagen, the content and arrangement of collagen with in tissue is a major factor in modification of the manner and extent to which a tissue attenuates the acoustic wave. The applications of USG in head and neck region is for examination of the thyroid gland, the salivary gland, the eye, examination of fetal face and sonically guided surgery.

Ian R Wilson (1989)[14] concluded that USG imaging of the superficial structures of the head and neck region plays a significant part in the investigation of virtually all non acute superficial swelling and mass lesions. USG have got added advantage of enabling clearer definition of the tissues of the head and neck, better documentation of the range of clinical application, assessment of the accuracy of USG in predicting subsequent histopathologic findings. They have recommended that USG should be a part of the diagnostic equipment used in oral radiology.

Jackowski J et al (1999)[47] compare the ultrasonographic (USG) appearances of the oral mucosa in health with systemic sclerosis and concluded that 2MHz sonography may be suitable as a non-invasive tool for evaluation of fibrosis of the oral mucosa.

Müller et al (1999)[48] conducted a clinical study in 33 patients to measure gingival thickness by means of USG. The transducer probe 4 mm in diameter was applied at the midfacial sites of each tooth, with light pressure to produce acoustic coupling. The result concluded that USG was demonstrated to be useful for gingival thickness measurement.

A study was attempted to investigate the reproducibility of the pulsed ultrasound technique for the determination of skin thickness using two independent observers. Studies were undertaken to validate the pulsed ultrasound technique as a measure of true skin thickness. Skin thickness was determined invitro (histometric analysis) was found to be greater than invivo (ultrasound) determination, probably due to release of invivo tension within the dermis after excision.

Kiliardis et al (2007)[49] studied the bilateral differences in the thickness of the masseter muscles in untreated individuals with lateral crossbite, as well as in subjects with successfully treated functional lateral crossbite, at least three years after the end of treatment. He reported that USG imaging has also been shown to be a useful tool to measure muscles thickness.

Serra et al (2008)[50] discussed the advantages and disadvantages of using ultrasonography to assess the masticatory muscles. The authors reported that there were different techniques available for recording the thickness of the muscles, and that the ultrasound technique generally showed lower reproducibility in relaxed than in contracted muscles. The authors suggested that ultrasound should be preferred in comparison with CT and magnetic resonance imaging due to its safety and cost advantages, since it is as reliable and precise as those techniques

Wakasugi-Sato et al (2010)[51] demonstrated the clinical applications of ultrasound imaging in soft tissue lesions, guided fine-needle aspiration, measurement

of tongue cancer thickness, and diagnosis of metastasis to cervical lymph nodes. The Doppler mode in ultrasound was reported to be a useful modality in the differential diagnosis between normal and metastatic lymph nodes in patients with oral squamous cell carcinoma.

Rangaiah et al (2010)[52] aimed to measure submucosal thickness by high frequency USG in cases and controls, to correlate this with clinical and histological severity of the OSMF. Cases had increased submucosal thickness when compared to controls at all measured sites. The echogenicity pattern of cases showed areas of irregular hyperechoic linear streaks due to fibrotic submucosal deposit. In contrast, the submucosa of controls appeared as a hypoechoic band.

Manjunath et al (2011)[53] conducted study to evaluate oral submucous fibrosis (OSMF) by clinical and histopathological examination, and compare the results with those from ultrasonographic technique. USG demonstrated number, length and thickness of the fibrotic bands. It helps in alteration of the treatment schedule in selected cases and allows for post treatment follow-ups and assessment. So, it could be a better diagnostic tool compared to clinical and histopathological examination.

Devathambi et al (2013)[54] evaluate the efficacy of USG in assessing the severity of OSMF and also to assess the relationship between OSMF and hypertrophy of massetter muscle. He concluded that USG is an effective non invasive tool to assess the progression of OSMF.

Krithika et al (2013)[55] characterize the ultrasonographic features of the buccal mucosa in patients with oral submucous fibrosis. Ultrasonography of the buccal mucosa demonstrates increased submucosal echogenicity and reduced echo differentiation between submucosa and muscle layer in OSMF cases. Hence, it can

be used as a non-invasive imaging modality to assess the disease extent and severity across the entire buccal mucosa to supplement clinical evaluation.

Joshi et al (2014)[56] reported that USG is easy to-use for the detection of non-invasive and soft tissue related diseases in oral and maxillofacial regions. USG plays an important role in analyzing normal and abnormal structures. In particular, in oral and maxillofacial regions, the USG may be clinically applied to evaluate lymph nodes, subcutaneous, and oral cavity-related diseases

PATHOGENESIS OF OSMF

Different hypotheses have been put forward so far in fully elucidating the pathogenisis of OSMF. The ***betel quid (BQ)*** chewing has been recognized as one of the important risk factors for OSMF as supported by the various experimental studies. The alkaloids and flavonoids from the BQ are absorbed and undergo metabolism which are the constant source of irritation to the oral mucosa during their contact. In addition, the fibres of areca nut also cause mechanical irritation to the oral mucosa which facilitates the diffusion of alkaloids and flavanoids into the subepithelial connective tissue, resulting in juxtaepithelial inflammatory cell infiltration[57].

Inflammation is characterized by the presence of activated T cells, macrophages and various chemical mediators. Persistent inflammation is crucial for the occurrence of tissue fibrosis. Thus, it can be considered that the induction of oral mucosal inflammation by BQ ingredients to be a critical event in the pathogenesis.

Growth factors like ***transforming growth factor- β (TGF-β)*** are synthesized at the sites of inflammation. At the molecular level, the collagen production and degradation are regulated by TGF-β and flavonoids present in areca nut[58].

Collagen production pathway:

The three main events which favours the collagen production are

1. Activation of procollagen genes

2. Elevation of procollagen proteinases levels

3. Upregulation of lysyl oxidase (LOX) activity

TGF-β activates the procollagen genes, resulting in the production of more pro-collagen. In OSMF, there is increased cross-linking of the collagen, resulting in increased insoluble form of collagen. The flavanoids also increase cross-linking in the collagen fibers.This is facilitated by increased activity and production of a key enzyme – LOX, which result in increased collagen production[59].

Collagen degradation pathway:

There are two main events regulated by TGF-β which decreases collagen degradation

1. Activation of tissue inhibitor of matrix metalloproteinases gene (TIMPs).
2. Activation of plasminogen activator inhibitor (PAI) gene.

TGF-β activates genes for TIMPs which inhibits the activated collagenase enzyme that is necessary for degradation of collagen. It also activates the gene for PAI, which is an inhibitor of plasminogen activator, results in absence of active collagenase. The flavanoids inhibit the collagenase activity. A reduction in the activity and levels of collagenase results in a decrease in collagen degradation[60].

Lysyl oxidase (LOX) is a copper activated enzyme critical for collagen cross-linking and organization of extracellular matrix (ECM), which has been shown to be ten times more resistant to digestion by collagenase. A study was conducted to compare the LOX activity of fibroblasts derived from human normal mucosa and OSMF associated with betel nut chewing. The study revealed that OSMF fibroblasts showed reasonably more lysyl oxidase activity than normal mucosa fibroblasts and this was statistically significant ($p<0.05$)[61].

Copper also has been implicated in the pathogenesis of OSMF. Areca nut has been found to have a high copper content and play an important role in the pathogenesis of OSMF. The possible role of copper functioning as a mediator of fibrosis in OSMF has been proved by the finding that raised copper levels in oral biopsies from patients with OSMF[62].

Plasminogen/plasmin system plays an important role in maintaining the equilibrium between synthesis and degradation of extracellular matrix (ECM). Plasminogen Activation inhibitor(PAI-1) inactivtes the plasminogen activators resulting in a decreased production of plasmin which is required for the degradation of ECM. Hence increased concentrations of PAI-1 leads to an accumulation of ECM[63].

OSMF is characterized by qualitative and quantitative alteration of collagen within the subepithelial layer of oral mucosa. The ***degradation of collagen by fibroblast phagocytosis*** is an important physiological remodeling of connective tissue. OSMF tissues exhibited 40% reduction of collagen phagocytic cells and a 48% decrease of fibronectin phagocytic cells as compared to normal fibroblasts. Normal fibroblast cultures incubated with areca nut alkaloids provided a dose-dependent reduction in the proportions of phagocytic cells. Thus inhibition of fibroblast phagocytosis by alkaloids provide a mechanism for the development of OSMF[64].

Further persistent tissue inflammation is thought to play a vital role on the occurrence of tissue fibrosis. The induction of oral mucosal inflammation by arecoline may be critical in the pathogenesis of OSMF. A study was conducted to compare role ***Cyclooxygenase (COX)-2*** expression in normal buccal mucosa and

OSMF patients. The study found that COX-2 expression was significantly higher in OSMF specimens compared to normal buccal mucosa, however when the cells were treated with 80 μg/ml arecoline, COX-2 expression was upregulated in normal mucosa. These results concluded that the upregulation of COX-2 expression in human buccal mucosal fibroblasts could play a vital role in the pathogenesis of OSMF[65].

Cytokines play an important role in regulating proliferation, migration and matrix synthesis of fibroblast and it is the balance of these mediators which play a key role in regulating the initiation and progression of any fibrotic disease. TNF-α, IL-1, IL-6 and IL-8 have been implicated in the development of fibrosis. Conversely, IFN-γ is an antifibrotic cytokine and downregulation of which is seen in keloid and scleroderma patients[66].

A hypothesis that is commonly reported in OSMF patients is the ***epithelial alteration***. The epithelium is considered to be "atrophic" and therefore vulnerable to the effects of oral carcinogens. "Atrophy" is explained to be arise as a result of stromal changes, which include decrease in cellularity and vascularity with resultant tissue ischemia and undergoes progressive hyalinization[67].

However the epithelium in OSMF failed to demonstrate an increased Absolute Cell Death Index (ACI) often seen in tissue atrophy. An alternative hypothesis was proposed which state that hypoproliferation of epithelium was a factor which causes thinning of surface epithelium rather than atrophy in advanced cases of OSMF[68].

Recent study with regard to vascularity in OSMF was conducted to assess the degree of expression of ***nitric oxide (NO)***, a net vasodilator, in OSMF. The study

concluded that enhanced expression of inducible nitric oxide synthase (iNOS) noticed in OSMF mucosa. NO has diverse properties of angiogenesis, vascular dilatation and increased permeability of vessels. These properties are all contrary to the concept of tissue hypoxia in OSMF and therefore the proposed "ischemic atrophy" of the overlying epithelium. This augments the earlier contention of an alternative explanation for thinning of the epithelium often noticed in clinically advanced cases. The thinning may be attributed to the defective replenishment of the desquamated epithelial cell pool probably due to decreased proliferation of the adult stem cell. Based on this, hypoplasia being a more reasonable concept explaining epithelial "thinning" than that of "atrophy". The possible genotoxic and cytotoxic effects of NO on adult stem cells of epithelium and supporting stroma supports further impetus to this concept[69].

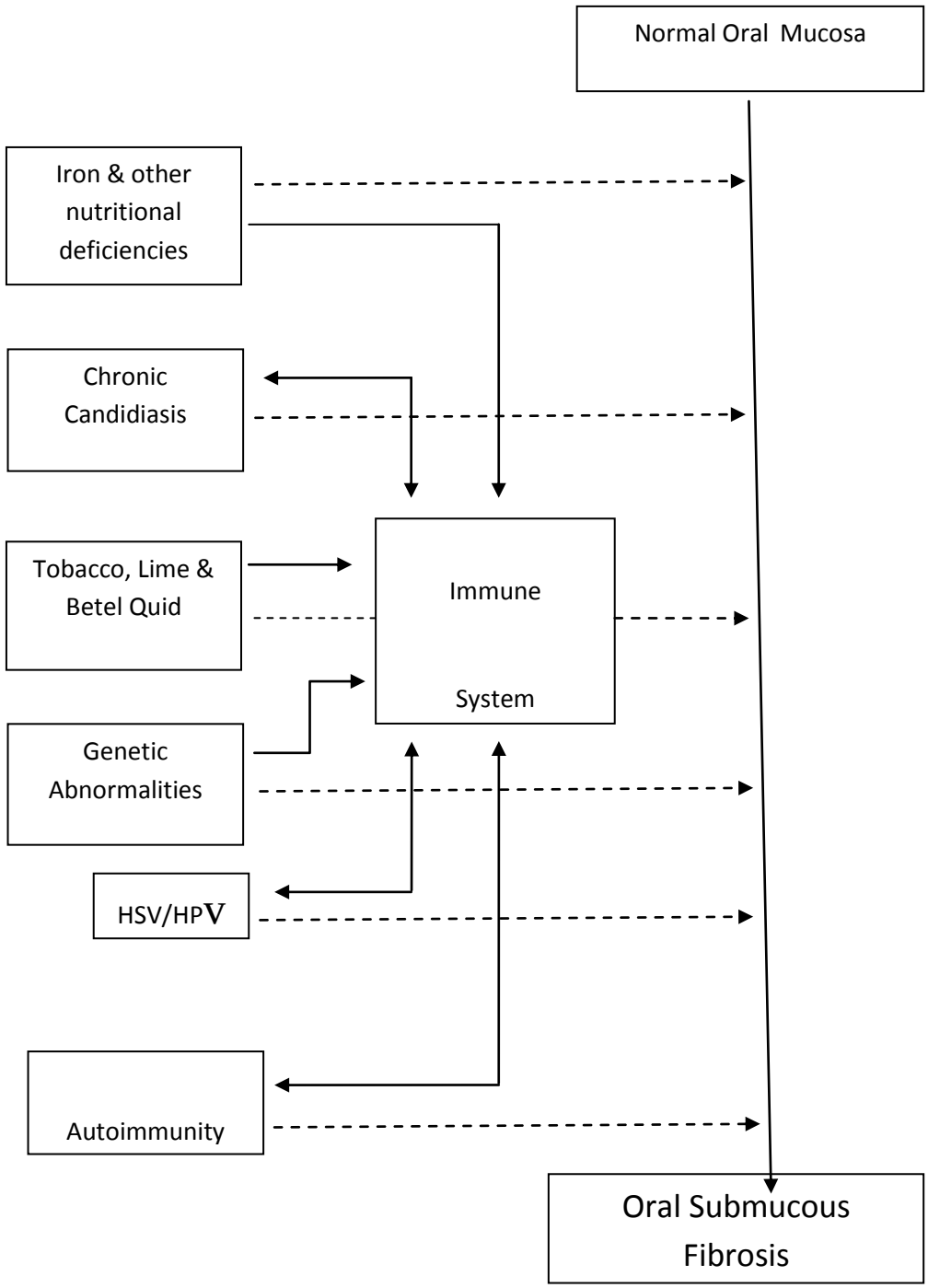
Regulation of ***transglutaminase-2 (TGM-2)*** by arecoline in oral fibroblasts have been found to play a major role in stabilizing the ECM proteins by cross-linking and making them highly resistant to protease degradation. This results in the accumulation of ECM, leading to fibrosis in OSMF cases. The expression of TGM-2 was studied in OSMF tissues by real-time RT-PCR analysis, and significant overexpression was observed in most OSMF tissues ($p=0.0112$) compared with normal tissues[70].

Currently the pathogenesis of OSMF have focused on ***heme oxygenase-1 (HO-I)*** expression in fibrosis. HO-I, a microsomal enzyme, responsible for maintaining the cellular homeostasis. It plays an important protective role in the tissues due to reducing oxidative injury and attenuating inflammatory response. HO-I is consistently and dramatically upregulated in a variety of fibrotic diseases, such as benign prostatic hyperplasia and cystic fibrosis of lung. OSMF demonstrated

significantly higher HO-I mRNA expression than normal buccal mucosa on immnuhistochemistry. Arecoline was also found to elevate HO-I mRNA expression in a dose-dependent manner[71].

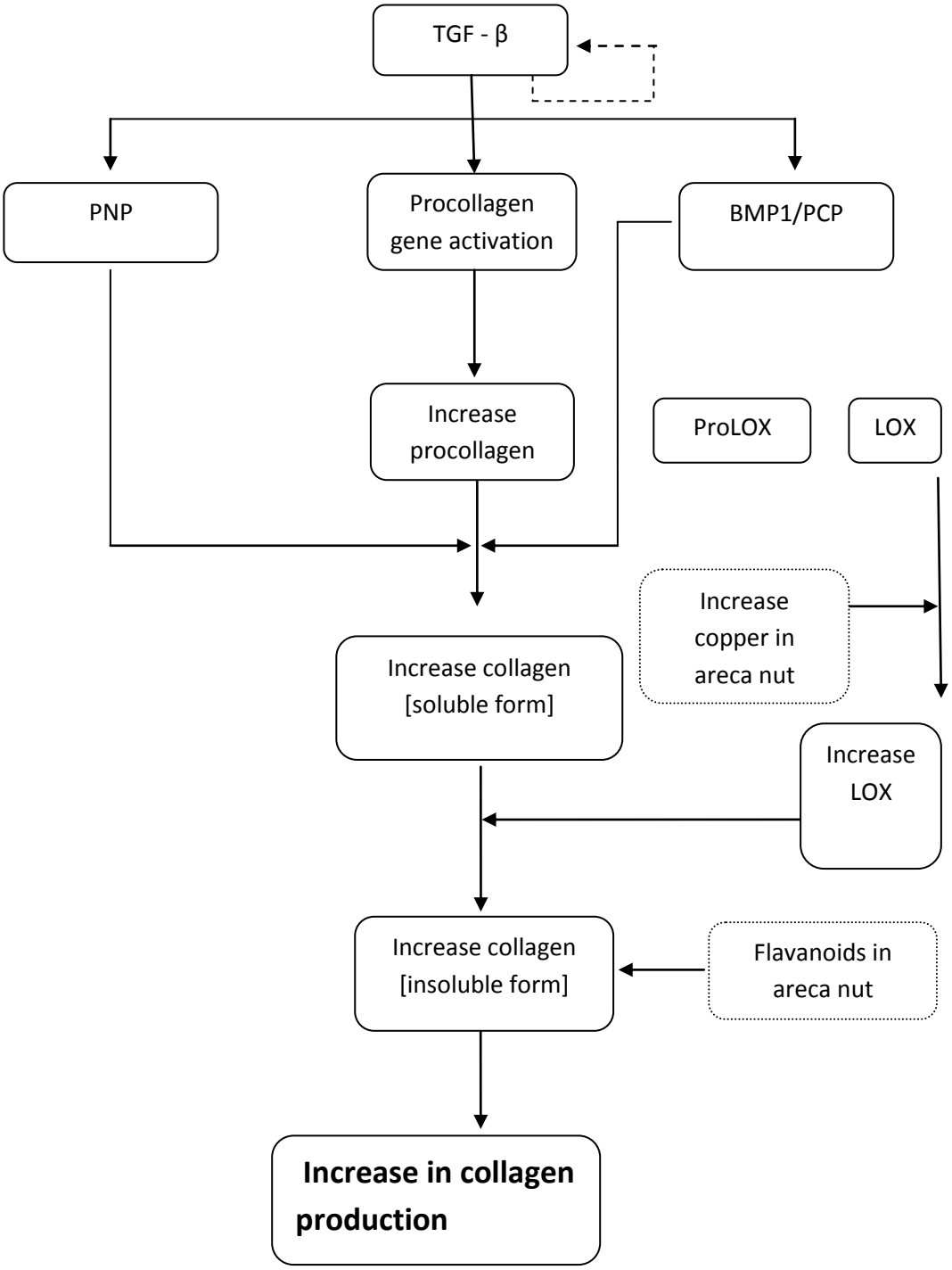
As OSMF produces changes localized to oral cavity, it has been put forth that ***saliva*** may have a role in the pathogenesis of OSMF. Saliva of OSMF patients have shown increased pH, increase in salivary amylase, increase in alkaline phosphatase and potassium, low level of calcium and normal level of salivary immunoglobulin. Formation of coagulum was observed in greater number of cases as the severity of the disease increased. It is thus postulated that the mechanical trauma due to chewing of betel nut, tobacco and chemical burns from slaked lime result in microhaemorrhage. The factor responsible for coagulum in saliva precipitates the increased laying down of fibroblast[72]. Increase in immunoglobulin levels is typically associated with three main chronic disease classes: those affecting the liver, collagen and chronic infections. The severity of OSMF was directly proportional to the estimated elevated levels of the major immunoglobulins IgG and IgA[73].

MULTIFACTORIAL MODEL FOR PATHOGENESIS OF OSMF

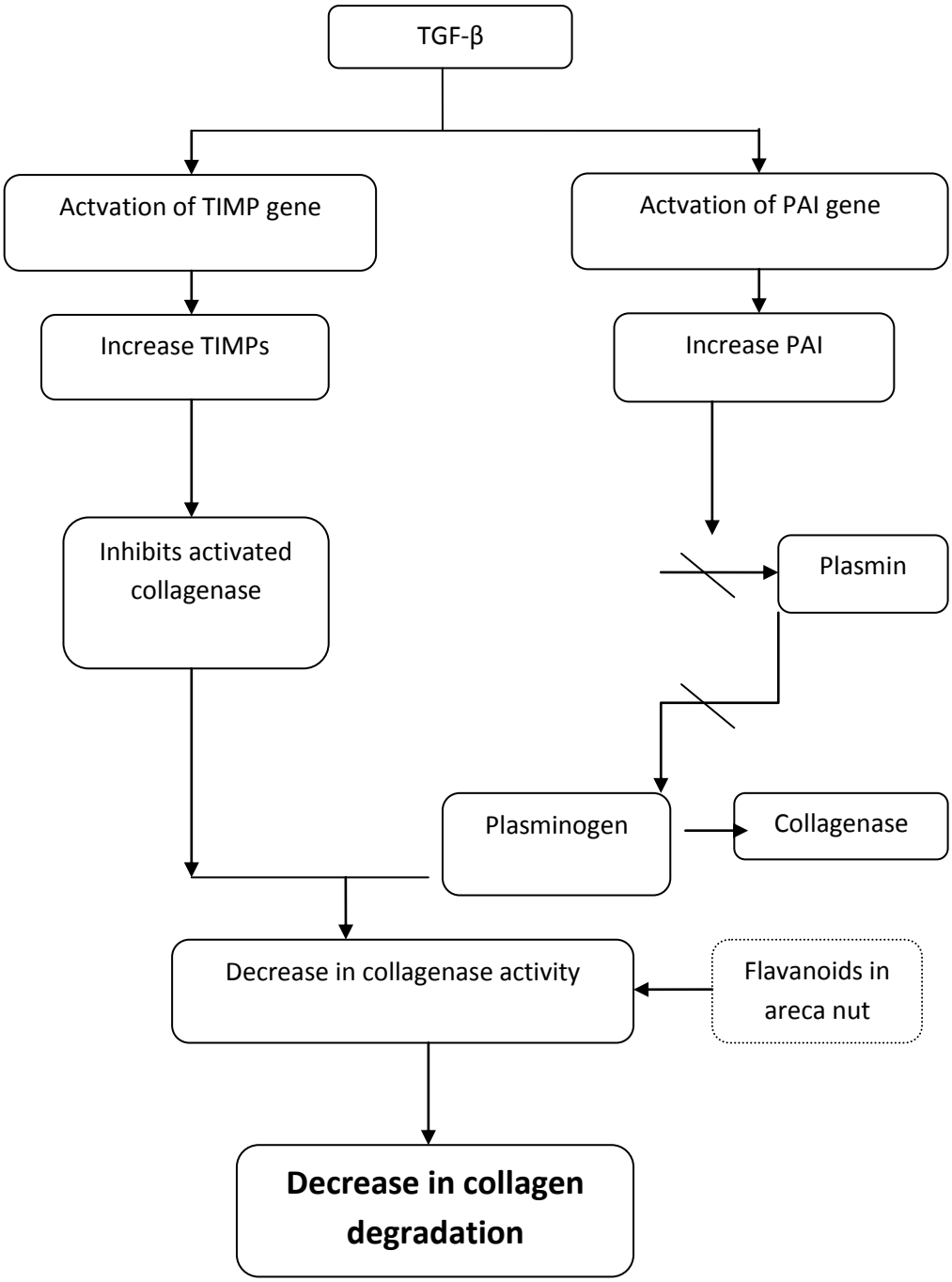


(Bold arrows show effects mediated by various factors through the immune system, whereas broken arrows show possible direct effects of the factors on the oral mucosa.)

COLLAGEN PRODUCTION PATHWAY



COLLAGEN DEGRADATION PATHWAY



PHARMACOLOGY OF PENTOXIFYLLINE

Pentoxifylline (oxpentifylline) is a tri-substituted methylxanthine derivative, chemically it is 1-(5-oxyohexyl)-3, 7dimethylxanthine, 1-(5oxyohexyl) theobromine.

MECHANISM OF ACTION

Pentoxifylline has potent rheologic modifying effects. Pentoxifylline and its metabolites blocks red cell aggregation, lower blood viscosity, inhibits microvascular constriction, and stimulates fibrinolysis. It also has significant anti-inflammatory, antifibrotic and immunomodulatory effects[74].

Effect on blood viscosity and flow

- Increased red cell deformability and aggregation
- Decreased circulating plasma fibrinogen
- Decreased vasoconstriction.

Immunologic effects

- Increased leukocyte deformability
- Decreased leukocyte adhesion and aggregation
- Decreased neutrophil superoxide release and degranulation
- Decreased neutrophil priming by platelet activating factor
- Increased leukocyte chemotaxis
- Decreased monocyte TNF-α production
- Decreased leukocyte response IL-1 production
- Decreased natural killer cell activity

Effect on coagulation and fibrosis

- Decreased platelet adhesion and aggregation
- Increased tissue plasminogen activator and plasmin

- Increased antithrombin III
- Decreased α_2- Antiplasmin and α_1 - Antitrypsin

Effect on wound healing and connective tissue

- Increased fibroblast collagenases
- Decreased fibroblast collagen and fibronectin
- Decreased fibroblast glycosaminoglycans
- Decreased fibroblast response to TNF-α

Pentoxifylline improves membrane deformability by increasing the amount of membrane ATP. It also alters red blood cell membrane protein phosphorylation patterns, increase protein kinase activity and decrease Ca^{2+} dependent K^+ efflux[75].

The mechanism of action of pentoxifylline in increasing polymorphonuclear cell chemotaxis is multifactorial. Pentoxifylline causes a dose dependent increase in cyclic adenosine monophosphate (cAMP) in polymorphonuclear cells. Cytoskeletal interactions are important in neutrophil adhesion, chemotaxis, phagocytosis and superoxide production. Pentoxifylline may act as an adenosine analogue, modulating cytoskeletal interactions. Pentoxifylline inhibition of lymphocyte activation also involves a cAMP dependent pathway[76].

The effect of pentoxifylline on decreased platelet aggregation can be explained on the basis of blocking of phosphodiesterase conversion of cAMP to AMP. Pentoxifylline stimulates vascular endothelium to release prostacyclin I_2, which further inhibits platelet adhesion and aggregation.**[116]** TNF-α has been implicated in disseminated intravascular coagulation by stimulating endothelial production of procoagulant tissue factor and decreasing endothelial thrombomodulin resulting in decreased protein C activation[77]. Some of the beneficial effects observed

with pentoxifylline therapy for hypercoaguable states may be related to its anti-TNFα effects.

The results of experimental studies have shown that fibroblasts cultured in the presence of pentoxifylline produced twice as much collagenase activity and decreased amounts of collagen, glycosaminoglycans and fibronectin. Interleukin-1 induced fibroblast proliferation is also inhibited by pentoxifylline[78].

PHARMACOKINETICS

Pentoxifylline is administered through oral and intravenous routes. The drug is almost completely absorbed after oral administration. It undergoes a first-pass metabolism and the various metabolites appear in plasma very soon after absorption. Intestinal absorption is rapid, with peak plasma concentrations obtained at 3.2 hours. The drug is metabolized by red blood cells and the liver, with an elimination half-life of 3.4 hours. There is extensive enterohepatic circulation. More than 90% of absorbed pentoxifylline is excreted in urine in the form of six metabolic products. Pentoxifylline and a seventh metabolite are not excreted in the urine of humans which have more pronounced physiologic effects than the primary drug[79].

DOSAGE AND ADMINISTRATION

The recommended adult dosage of oral pentoxifylline is 400mg thrice daily with meals. Doses as high as 2200mg daily have been well tolerated in patients with severe peripheral vascular disease. Duration of 3 to 12 months may be required for noticeable improvement in fibrotic skin disorders or other fibrosis[80].

Intravenous pentoxifylline is also available. Slow intravenous infusion beginning with 100mg/day is recommended with a daily increase in increments of 50 mg. Although the maximum recommended adult dose is 300mg/day, doses as high as

600g twice a day have been used successfully to treat gangrenous arterial occlusive disease[81].

PREPARATIONS COMMONLY AVAILABLE:

OxpentifyllineTab-400mg TNMSC (Tamilnadu Medical service corporation)

Trental Tab-400mg, (Sanofi-Aventis)

ESTABLISHED CLINICAL APPLICATIONS[74]

Pentoxifylline being a FDA approved drug and is currently used for various disorders in the medical field without significant complications.

Vasoocclusive disorders:

- Peripheral vascular disease
- Cerebral vascular disease
- Diabetic vascular disease
- Polycythemia vera
- Ischemic heart disease
- Chronic renal insufficiency

Hypercoagulable states

- Post operative thrombotic complications
- Transient ischemic attacks

ADVERSE EFFECTS [82]

Most side effects caused by pentoxifylline involve the gastrointestinal tract and central nervous system. Nausea, vomiting, indigestion, gastric irritation, headache, dizziness are the commonly reported side effects.

DRUG INTERACTIONS

Patient on oral anticoagulant are at higher risk of prothrombin time elevations when combining with pentoxifylline. Hence patients on warfarin should have more

frequent monitoring of prothrombin time. Patients with other risk factors like hemorrhage (e.g.,recent surgery, peptic ulceration) should have periodic examinations for bleeding including hematocrit and/or hemoglobin.

Combination of pentoxifylline and theophylline should be avoided which leads to increased serum theophylline levels and toxicity in some individuals[83]. Pentoxifylline has been used concurrently with antihypertensive drugs, beta blockers, digitalis, diuretics, antidiabetic agents, and antiarrhythmics, without observed problems.

Small decrease in blood pressure has been observed in some patients treated with pentoxifylline, periodic systemic blood pressure monitoring is recommended for patients receiving concomitant antihypertensive therapy. If indicated, dosage of antihypertensive agents should be reduced.

CONTRAINDICATION

Pentoxifylline should not be used in patients with recent cerebral or retinal hemorrhage or in patients who have previously exhibited intolerance to the drug or other methylxanthines such as caffeine, theophylline and theobromine.

PENTOXIFYLLINE AND OSMF

Pentoxifylline with potent hemorrheologic properties has been proved to be effective in treating intermittent claudication[84]. It was therefore convicted that it might also be effective in treating OSMF which having mucosal ischemia and epithelial atrophy. The submucosal fibrosis, which is the hallmark of this disorder, is considered to be the result of a defective inflammatory reparative response resulting in fibrotic healing[85].

The anti-inflammatory and immunomodulatory actions of pentoxifylline seems to have definite therapeutic implication in the management of OSMF. As

OSMF is a chronic inflammatory disease, control of the inflammation or the factors influencing the inflammatory changes should form the basis of definitive management. Pentoxifylline has the ability to decrease the production of tumour necrosis factor alpha (TNF-α), an important mediator of the inflammatory process[86].

Primary immunologic abnormalities have also been reported in OSMF which probably mediate local tissue damage. The immune-modulating actions of pentoxifylline include decreased leukocyte adhesion, aggregation and degranulation, decreased superoxide release and decreased natural killer cell activity[87].

OSMF is considered to be a collagen metabolic disorder with abnormal accumulation of collagen in subepithelial layers. An increased production and a reduced degradation of the type I collagen have been observed in OSMF. Collagenases are the only proteinases which specifically cleave triple helical collagen at neutral pH. A reduced content of functional collagenase observed in OSMF might be one of the mechanisms responsible for this collagen accumulation. Fibroblasts cultured in the presence of pentoxifylline produce twice as much collagenase activity and decreased amount of collagen, glycosaminoglycans and fibronectins[88].

Cytokines play an important role in regulating fibroblast function, such as proliferation, migration and matrix synthesis, hence it is likely to play a key role in regulating the initiation and progression of any fibrotic disease. Both interleukin-1 (IL-1) and tumour necrosis factor alpha (TNF-α) stimulate fibroblast proliferation in vitro and intradermal injections of TNF-α stimulate the accumulation of fibroblasts and collagen. Similarly, both IL-6 and IL-8 have been implicated in the development of fibrosis. Studies conducted in OSMF `patients have shown increased levels of proinflammatory cytokines: IL-1, IL-6, IL-8 and TNF-α and reduced anti-fibrotic

cytokine interferon gamma (IFN-γ) which may be central to the pathogenesis of OSMF. Interleukin -1 induced fibroblast proliferation was inhibited by pentoxifylline. In addition, pentoxifylline blocked the TNF-α induced synthesis of fibroblast collagen, glycosaminoglycans and collagenolytic activity[89].

A randomized clinical trial was conducted to determine the effects of pentoxifylline on the clinical and pathological course of OSMF. The results showed highly significant improvement in mouth opening (p=0.000), tongue protrusion (p=0.002), relief from perioral and buccal mucosal fibrotic bands (p=0.000), burning sensation of mouth (p=0.0005), tinnitus (p=0.00004), difficulty in swallowing (p=0.00007) and speech (p=0.000) in all the test subjects. Adverse effects reported were mild gastritis and gastric irritation, peripheral flushing that could be managed easily. The study concluded that pentoxifylline is an effective adjunct therapy in the routine management of OSMF[43].

PRINCIPLES OF ULTRASONOGRAPHY

An image is a reproduction, representation, or imitation of the physical form of a person or thing. An ultrasound image is the visible counterpart of an invisible object, produced by an electronic instrument. Ultrasound provides a non-invasive way of looking inside the human body to image otherwise unseen anatomy. Anatomic imaging with Ultrasound is accomplished with a pulse echo technique.

Ultrasound is defined as the sound above the range of human hearing i.e., above the frequency of 20,000 Hz (normal human hearing is between 20 Hz – 20,000 Hz). Ultrasonic waves require a medium for their propagation, since they cannot travel in vacuum. The use of ultrasound to obtain diagnostic information is called Ultrasonography. Medical use of ultrasound involves frequencies greater than 3 MHz.

ULTRASOUND PRODUCTION:

In an ultrasound machine, ultrasound is produced by passing electric current through Piezo – electric crystals (PZE) contained in its "transducer" portion. PZE crystals have the unique property of "interconverting" various forms of energies as follows. All PZE crystals possess" large" loosely held charged atoms which shift in response to the applied electrical field which in turn leads to the following two changes

a. Change in shape of crystals with liberation of mechanical energy.

b. Generation of vibration with liberation of sound energy.

The reflected ultrasounds (echoes) are collected by the transducer (serves as a transmitter and receiver at the same time), in a reverse order than that of the piezoelectric effect, the sound waves are converted to mechanical energy first, and

then to an electrical charge. Because of its acoustic impedance, a tissue has a characteristic internal echo pattern or echogenicity. Consequently, not only changes in the echo patterns delineate different tissues, but they can also be correlated with pathologic changes in a tissue[90].

TRANSDUCERS:

Pulses of ultrasound are generated by a transducer and are sent into the patient, where they produce echoes at organ boundaries and within tissues. These echoes then return to transducer, where they are detected and then presented on the display of a sonographic instrument. A transducer converts one type of energy into another. Based upon the pulse-echo principle occurring with ultrasound piezoelectric crystals, ultrasound transducers convert: [17]

- Electricity into sound = pulse
- Sound into electricity = echo

Mechanism Of Conduction Of Ultrasound Through Tissues[91]:

Ultrasound energy is transformed through tissue from "particle to particle and runs in the longitudinal wave form. The ultrasound energy which is passes to the tissue is directly related to the frequency of ultrasound energy used. The Ultrasonic waves are reflected and refracted at the boundary where two types of tissues with different acoustic impedance exist adjacent to each other. The acoustic impedance in bone is greater than that in soft tissues and therefore most of ultrasound waves reflect at the surface of bone but reflects very little from the inside of bone. A tissue such as the surface of bone is visualized as hyperechoic area.

ECHO MECHANISM OF ULTRASOUND:

Echo generation : Ultrasound beam as it passes through various interphase of tissue gets partially reflected back from each of the interphase which constitutes the echo which can be specular or scattered.

Echo – Receiving Mechanism: In ultrasound transducer (probe) the arrangement is such that the same PZE crystals which produce ultrasound beam (on being stimulated by electric current) also serve to receive the returning ultrasound energy (echo) from the tissue.

PZE crystals on receiving the echo convert it into "electrical energy" by its inherent property, which is then converted into high energy by the ultrasound machine, which is then visualized as dots of light constituting a picture on a monitor.

Chemistry of PZE Crystals:

Ceramic: Quartz, barium, titanium (most commonly used). Being ceramic they are very fragile and hence ultrasound transducers must be handled with great care.

Plastic: Lately they are being synthesized and may allow a little rougher handling. These PZE crystals are coated as "thin plate" in the shape of a disc in the transducer.

Metal electrode material is then deposited on both of its surface electrolytically. Application of voltage across the electrode causes the plate to vibrate to produce ultrasound. The frequency of ultrasound produced is directly proportional to the thinness of the plate, i.e. thinner the plate, the higher the frequency of ultrasound[92].

Different modes in ultrasound:

A – Mode (amplitude mode) is the simplest type of ultrasound. A single transducer scans a line through the body with the echoes plotted on screen as a function of depth. Therapeutic ultrasound aimed at a specific tumor or calculus is also A-mode, to allow for pinpoint accurate focus of the destructive wave energy.

B - Mode (Brightness modulation): Two dimensional display of the static image. The "B" mode produces a picture of a slice of tissue. Echoes are displayed as dots.

C-mode: C-mode image is formed in a plane normal to a B-mode image. A gate that selects data from a specific depth from an A-mode line is used; then the transducer is moved in the 2D plane to sample the entire region at this fixed depth. When the transducer traverses the area in a spiral, an area of 100 cm^2 can be scanned in around 10 seconds

M – Mode (motion mode): also called as real time scan. A real time system can produce multiple frames in a very short time. This fast frame rate allows movement to be viewed in "real time" as the images are generated.

D-mode (D=Doppler) This imaging mode is based on the Doppler effect, ie. change in frequency (Doppler shift) caused by the reciprocal movement of the sound generator and the observer. Diagnostic ultrasound uses the change in frequency of ultrasound signal backscattered from red blood cells. The frequency of the reflected ultrasound wave increases or decreases according to the direction of blood flow.

Gray Scale : It is a scale for quantification of echo signals which is nearest to help the interpretation of an ultrasound image. In gray scale imaging, strong echoes are displayed as light gray or white and weak echoes as dark gray or black. Between these, various shades of gray are displayed.

ADVANTAGES:

- It's a non invasive modality.
- It images muscles and soft tissues very well and is particularly useful for delineating the interfaces between solid and fluid filled spaces.
- It shows the internal structure of the organs.
- No known long term side effects and early causes discomfort on pain to the patient.
- Renders line images, where the operator can dynamically select the most useful section for diagnosing and documenting changes often enabling rapid diagnosis
- Equipment is widely available and is comparatively flexible.
- Small easily carried scanners are available and examinations can be easily preformed at the bed side.
- Relatively inexpensive compared to CT & MRI. [17]

LIMITATIONS:

- Obese patients limit image quality as the overlying adipose tissue scatters the sound and greater depth the sound waves need to travel, weaken the signal on transmission reflection back to the transducer.
- Ultrasound performs very poorly when there is gas between the transducers and organ of interest due to extreme differences in acoustical impedance.
- Ultrasound devices have trouble in penetrating bone.
- Once an image is acquired there is no exact way to tell which part of the body was imaged.
- Method is operator dependent high level of skill and experience is needed to acquire good quality images and make accurate diagnosis

USG is also a method for measurement of oral mucosa, which has been applied for measurement of gingival thickness in attached gingival, palatal mucosa, masticatory muscle thickness[48,50].

CLINICAL APPLICATIONS OF ULTRASONOGRAPHY IN ORAL MUCOSA:

The echogenecity of a tissue primarily relates to its "stiffness", the chief source of which is collagen, the content and arrangement of collagen with in tissue which is a major factor in modification of the manner and extent to which a tissue attenuates the acoustic wave. The applications of USG in head and neck region is for examination of the thyroid gland, the salivary gland, examination of fetal face and sonically guided surgery.

USG provides both qualitative and quantitative assessment. Qualitatively it provides information on the nature of the swelling or lesion, whether it is soild or cystic, whether calcifications present, and relations of lesion to adjacent normal structures. Quantitatively it may assess the dimension of the lesion, its distance from the skin surface and its relative proximity to skin and mucosal surfaces -delineation of the mucosal surface often being aided by placement of a finger on that surface adjacent to field of view. Measurements are obtained atraumatically, rapidly and rather inexpensively. Suspect measurements may be repeated immediately, checked and averaged. Ultrasonic assessments of mucosal thickness in different parts of oral cavity may depend on the difficulties of repeatedly measuring at the same location, on varying thickness of tissue. These problems might be resolved by averaging multiple measurements. They also concluded that USG device yielded valid and relatively reliable information on thickness of most parts of masticatory mucosa.

MATERIALS AND METHODS

The study was conducted after getting approval from the Institutional Ethical Committee.

STUDY CENTRE:

Department of Oral Medicine and Radiology,

Tamil Nadu Government Dental College and Hospital,

Chennai – 600 003.

Bernard Institute of Radiology,

Rajiv Gandhi Government Hospital,

Chennai -600003.

CASE SELECTION:

The study population include total of 30 patients, either of the sex who were diagnosed as OSMF based on habitual history and clinical findings which include the presence of burning sensation in the mouth, blanching and loss of stiffness of oral mucosa, presence of fibrous bands and restricted opening of mouth. Then the patients were selected who satisfying the following inclusion and exclusion criteria.

Inclusion criteria:

- Patient with age group of 20 to 60 years, both Gender.
- Patients who satisfy the characteristic clinical features of OSMF
- Patients who are not taking any medication for their disease condition.

Exclusion criteria:

- Patients with chronic systemic diseases like cardiac, cerebrovascular, Respiratory, Renal, Hepatic, Gastrointestinal complications, collagen disorders, infectious diseases, bleeding disorders and diabetes mellitus .

- Patients with severe restricted mouth opening (<15mm)
- Pregnancy and lactation
- Patient intolerance to caffeine and theophylline
- Patients not willing to participate in the study.

METHODOLOGY:

Study subjects of 30 patients were included in the study based on inclusion and exclusion criteria, and those who satisfied the clinical criteria of OSMF. All the participants were explained about the need and design of the study, the drug therapy and their possible adverse effects (including gastric irritation, nausea, vomiting, dizziness), the need for thorough clinical examination, routine investigations and USG as a part of the study. Patients who gave a signed informed consent on an institutionally approved document were included in the study. Patient also subjected to routine blood investigation and Tobacco Cessation Counselling (TCC) in our institution before the start of the study and TCC were continued during the progress of the study.

A detailed case history of the patient with emphasis on their habits (chewing betel nut, pan parag, etc.) and a thorough clinical examination was recorded on a structured proforma designed for the study. A clinical diagnosis of OSMF was made and patients were numbered serially as they entered the study. 30 subjects were included conveniently in the study in single blind randomised manner to the following groups alternatively irrespective of age, sex and stage of OSMF.

GROUP A: Pentoxifylline group

GROUP B: Dexamethasone group

Then patients were graded clinically after assigned to the group, according to **Khanna JN, Andrade NN (1995)**[93]**,**

Group* I: Very early cases:*

Normal mouth opening

Burning sensation in the mouth,

Acute ulceration and recurrent stomatitis.

Group II: **Early cases**

Interincisal distance of 26 to 35 mm.

Buccal mucosa appears mottled and marble like,

Widespread sheets of fibrosis palpable and red erythematous patches

Group III: **Moderately advanced cases**

Interincisal distance of 15 to 25 mm,

Buccal mucosa appears pale firmly attached to underlying tissues,

Atrophy of vermilion border,

Vertical fibrous bands palpable at the soft palate, pterygomandibular raphe and anterior faucial pillars.

Group IVA: **Advanced cases**

Severe trismus,

Interincisal distance of less than 15 mm,

Restricted tongue movement,

Presence of circular band around entire lip and mouth.

Thickened faucial pillars, shrunken uvula

Group IVB : Advanced cases with premalignant and malignant changes

Oral submucous fibrosis and leukoplakia

Oral submucous fibrosis and squamous cell carcinoma.

Armamentarium:

Examination of the patient:[Figure 1]

- Electrically operated dental chair
- Patient's apron
- Disposable mouth mask
- A pair of disposable latex examination gloves
- Stainless steel kidney trays
- Mouth mirror
- Stainless steel probe
- Tweezer
- Divider and Metallic scale

Drug administration:[Figure 2]

- Sterile 3 ml disposable syringe
- Tablet oxpentifylline 400mg
- Topical local anesthetic gel
- Injection Local anaesthesia
- Injection dexamethasone 2ml vial
  - Injection hyaluronidase 1500 IU (HYNIDASE- *shreya pharma*)

All the drugs are supplied by TNMSC except HYNIDASE .

Ultrasonographic evaluation:[Figure 3 & 4]

- Philips high frequency ultrasound machine (3 – 12 MHz)
- Linear Transducer measuring 4 X 1 cms
- Acoustic coupler (Coupling agent)

  (Carbomer–10g, EDTA-.25g, Propyleneglycol-75g, Trolamine -12.5g)

COLLECTION OF DATA:

Patients were made to sit comfortably on a dental chair. Clinical examination was carried out wearing sterile hand gloves and mouth mask under artificial illumination including blanching of mucosa [Figure 5-7] and fibrous band palaption. Brief medical history was taken to rule out any possible systemic illness.

Clinical parameters included in the study to evaluate the effectiveness of the drugs were burning sensation and mouth opening. USG parameters included were submucosal thickness and echogenicity.

The intensity of **burning sensation** was determined using a Visual Analogue Scale (VAS) of 0-10 with 10 mm division, where 0 was no burning sensation and 10 was worst possible burning sensation. The patients were asked to mark VAS at a point which best represented their level of symptoms. The score was recorded at each subsequent visit after the administration of the drug therapy.

The **interincisal mouth opening** was measured using divider and scale from the mesio-incisal angle of upper central incisor to the mesio-incisal angle of lower central incisor and recorded in centimetres [Figure 8]. If the corresponding teeth were not present contra lateral teeth or adjacent teeth will be considered. Intraorally, different sites were examined for blanching of the mucosa, consistency, fibrous bands, and for presence of other lesions.

Complete haemogram and random blood sugar examination was done for all patients, then subjected to pre operative USG evaluation. Oral prophylaxis was carried out in all the patients before USG evaluation.

ULTRASONOGRAPHIC EVALUATION:

Ultrasonographic measurements of submucosal thickness and echogenicity were performed for 30 subjects comprising of 15 in each groups. Scanning was performed with the patient in supine position. The patients were positioned such that their head was in level with the examiners knees. The examiner was sat on the right side of the patient where the ultrasound apparatus was also placed. The ultrasonic transducer was cleaned by an antiseptic solution using surgical spirit. Coupling agent was applied on the linear transducer and also on the area of interest. Ultrasound examinations were performed by a single trained general Radiologist.

Transcutaneous imaging was done using ENVISOR CHD (Philips company) with multifrequency linear transducer with a frequency ranging from 3-12 MHz which was connected to the scanner. Transducer measures about 4 cms in length and 1 cm in width. With focal depth of approximately 6mm- 1cm.And a real time imaging of buccal mucosae was performed. Buccal mucosa was selected for USG evaluation as it is commonly involved next to faucial pillar and it is easily approachable for proper evaluation.

The transducer probe was placed in such a way that the soft tissues were not unduly compressed, because excess contact pressure while imaging might affect the measurements. Hence, to get the exact thickness measurements the probe was brought softly onto contact with the surface.

Patients were prior instructed to indicate the mucosa by placing the forefinger inside the mouth against the mucosa to delineate the lining mucosa and empty space of the oral cavity as stated by **Wilson et al**[14]**.** He also told that to assess distance from the skin surface and its relative proximity to skin and mucosal surfaces, delineation of the mucosal surface can often being aided by placement of finger on

the surface adjacent to field of view [Figure 10]. Patients were also told not to apply finger pressure against the mucosa but just a finger touch movement of the mucosa. For imaging the right buccal mucosa, patients left forefinger was used as an indicator and for left buccal mucosa corresponding right forefinger was used as an indicator. Once the patient moves the indicator finger against the mucosa a corresponding movement of mucosal lining was visualized on the monitor.

The real time imaging of submucosa of right and left buccal were carried on and the measurements were taken. Mucosal lining was seen as a hyperechoic linear line, submucosa as a band of hypoechoic zone supported by a muscle planes in controls. This band of hypoechogenicity in between hyperechoic mucosa and muscle layer was measured as submucosa. All measurements were taken in centimetres [Figure 11 & 12]. With increased severity of OSMF submucosa is thought to become more hyperechoic compared to hypoechoic in normal patients. Hyperechogenicity is marked as 1 and hypochogenicity is marked as 2. Since differences in reliability of ultrasonic assessments of mucosal thickness in different parts of oral cavity may depend on the difficulties of repeatedly measuring at the same location, on varying thickness of tissue. These problems might be resolved by averaging multiple measurements.

METHOD OF DRUG ADMINISTRATION:

After pre-operative USG evaluation, patient in Group A were administered oral pentoxifylline 400mg thrice daily after meals for 3 months and patient in group B were administered intralesional injection of 0.5ml of local anaesthesia with 2ml of dexamethasone and 1500 I.U of Hyaluronidase Bi-weekly for 6 weeks [Figure 9].

Patient in Group A were recalled and evaluated for improvement in signs and symptoms for every 3 days for first month and then every week for next two months.

Final changes in burning sensation and mouth opening were recorded after the complete course of treatment. Similarly patient in Group B were evaluated during every injection and values were recorded after the end of the course of injection.

POST OPERATIVE USG EVALUATION:

After the course of the treatment scheduled, all the patients of both the groups were subjected to post operative USG evaluation of submucosal thickness and echogenicity similar to pre-operative evaluation and the values are recorded. All the values were statistically analysed and the results were drawn.

PHOTOGRAPHS

FIGURE 1 : DIAGNOSTIC INSTRUMENTS

FIGURE 2: DRUGS USED IN THE STUDY

FIGURE 3 : ULTRASOUND MACHINE

FIGURE 4: COUPLING AGENT AND TRANSDUCER

FIGURE 5: BLANCHING OF BUCCAL MUCOSA

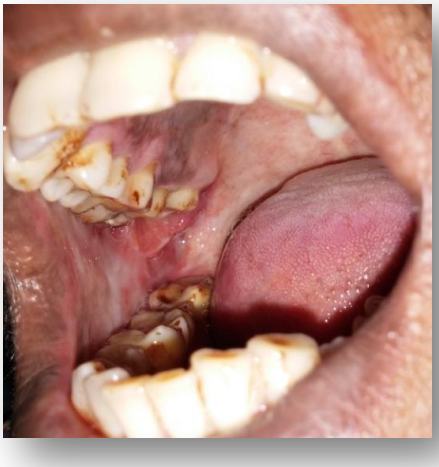


FIGURE 6 : BLANCHING OF LABIAL MUCOSA

FIGURE 7 : BLANCHING OF SOFT PALATE

FIGURE 8:MOUTH OPENING MEASUREMENT

FIGURE 9:INTRALESIONAL INJECTION THERAPY

FIGURE 10 : POSITIONING OF PATIENT FINGER AND TRANSDUCER

FIGURE 11 : USG EVALUATION - PRE OPERATIVE

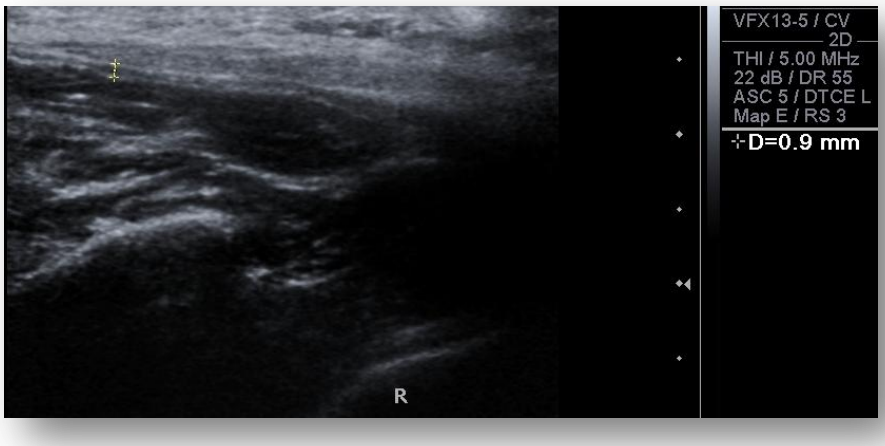
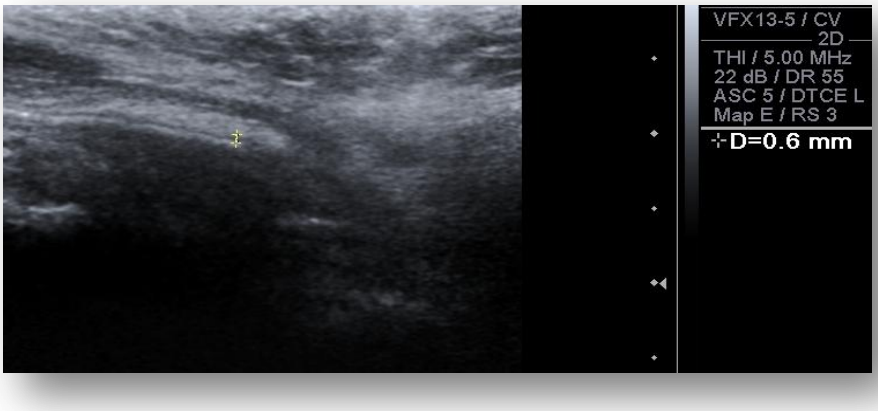


FIGURE 12: USG EVALUATION – POST OPERATIVE



MASTER CHART : GROUP A – PENTOXIFYLLINE

S.NO	AGE	SEX	BURNING SENSATION		MOUTH OPENING (mm)		STAGING	USG SUBMUCOSAL THICNESS (mm)				SUBMUCOSAL ECHOGENICITY (HYPER -1 : HYP0 -2)			
			PRE OP	POST OP	PRE OP	POST OP		PRRE OP		POST OP		PRRE OP		PRRE OP	
								RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT
1	23	M	7	0	25	29	III	0.63	0.54	0.45	0.36	1	1	2	2
2	29	M	5	0	35	41	II	0.48	0.46	0.29	0.26	1	2	2	2
3	32	F	6	0	23	28	III	0.47	0.63	0.34	0.41	2	1	2	2
4	36	M	7	0	20	24	III	0.83	1.12	0.64	0.84	1	1	2	2
5	25	M	10	0	19	23	III	1.06	0.98	0.83	0.76	1	1	1	1
6	29	F	5	0	22	25	III	0.61	0.87	0.38	0.63	1	1	2	1
7	45	M	10	0	26	29	II	0.65	0.64	0.45	0.44	1	1	2	2
8	38	M	3	0	24	31	III	0.78	0.65	0.52	0.45	1	1	2	2
9	47	M	5	0	29	34	II	0.65	0.75	0.46	0.52	1	1	2	2
10	36	M	10	0	26	29	II	0.85	0.55	0.53	0.33	1	2	2	2
11	29	M	7	0	24	29	III	0.61	0.87	0.42	0.63	2	1	2	2
12	33	F	2	0	23	27	III	0.68	0.83	0.41	0.61	1	1	1	2
13	32	M	5	0	21	24	III	0.89	0.78	0.62	0.51	2	1	2	2
14	43	M	8	0	32	38	II	0.64	0.63	0.41	0.39	2	1	2	2
15	54	M	10	0	35	42	II	0.65	0.67	0.48	0.46	1	1	2	2

MASTER CHART : GROUP B – DEXAMETHASONE WITH HYALURONIDASE															
S.NO	AGE	SEX	BURNING SENSATION		MOUTH OPENING (mm)		STAGING	USG SUBMUCOSAL THICNESS (mm)				SUBMUCOSAL ECHOGENICITY (HYPER -1 : HYP0 -2)			
			PRE OP	POST OP	PRE OP	POST OP		PRRE OP		POST OP		PRRE OP		PRRE OP	
								RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT	RIGHT	LEFT
1	32	M	5	0	31	33	II	0.66	0.68	0.53	0.54	1	1	2	2
2	28	M	7	0	25	28	III	0.66	0.93	0.68	0.77	1	2	1	1
3	25	F	3	1	16	18	III	1.06	0.83	0.93	0.66	1	1	1	2
4	43	M	8	0	28	31	II	1.03	0.96	0.88	0.82	1	1	1	1
5	35	M	10	0	30	32	II	0.67	0.43	0.49	0.32	2	1	2	2
6	39	M	6	0	18	21	III	0.93	1.08	0.82	0.96	1	1	1	1
7	27	M	5	0	29	33	II	0.92	0.74	0.78	0.61	1	2	1	2
8	36	F	7	0	19	21	III	0.88	0.73	0.74	0.59	1	1	1	1
9	42	M	3	1	35	38	II	0.84	0.74	0.71	0.61	1	1	2	2
10	29	M	10	0	26	29	II	0.44	0.46	0.32	0.33	2	1	2	2
11	23	M	6	0	30	32	II	0.62	0.64	0.54	0.55	1	1	2	2
12	26	M	7	0	25	29	III	0.66	0.68	0.53	0.55	1	1	2	2
13	43	M	4	0	32	34	II	0.52	0.55	0.42	0.41	1	1	2	2
14	38	F	9	0	33	36	II	0.45	0.54	0.36	0.38	2	1	2	2
15	26	M	8	0	31	34	II	0.72	0.65	0.58	0.53	1	1	1	2

STATISTICAL ANALYSIS

The statistical analysis was done using the computer software program SPSS version 18.

The percentage of distribution of age groups, sex was calculated within each group and overall distribution was also calculated. The overall distribution of stages of osmf was calculated among the study population. Distribution of burning sensation, mouth opening and submucosal thickness was also calculated in relation to stages.

Arithmetic mean and standard deviation were estimated for different variables in each study group**.**

Independent sample test were used to analyse association of staging with different vaiables used in this study. Paired sample test were used to analyse variables within each group. Independent t test were used to analyse variable between groups. Pearson Co-relation test were used to find correlation between mouth opening and submucosal thickness.

In the present study, $p < 0.05$ was considered as the level of significance.

TABLES

TABLE 1: DEMOGRAPHIC DISTRIBUTION:

		GROUP A	GROUP B
AGE (YEARS)	MEAN AGE	35.4	32.8
	RANGE	23-54	23-43
SEX (N=30)	MALE	12	12
	FEMALE	3	3

TABLE 2: PREVALENCE OF STAGE:

	GROUP A(n=15)	GROUP B (n=15)	overall (n=30)
STAGE II	6 (40%)	10(66.6%)	16 (53.3%)
STAGE II	9 (60%)	5 (33.3%)	14(46.6%)

TABLE 3: ASSOCIATION BETWEEN STAGE AND VARIABLES

	BURNING SENSATION(mean VAS score)	MOUTH OPENING (mm)	USG SUBMUCOSAL THICKNESS (mm)
STAGE II	7	29.4 (26-36)	0.66
STAGE III	6.6	21.2(16-25)	0.82
p value	.234	.000**	.006

****** - Highly significant

TABLE 4: COMPARISION OF BURNING SENSATION BETWEEN GROUPS

		PRE-OPERATIVE		POST-OPERATIVE		
GROUP	No. of patients	Mean	standard deviation	Mean	standard deviation	p value
GROUP A	15	6.66	±2.58	0.00	±0.00	0.000**
GROUP B	15	6.53	±2.26	0.13	±0.35	0.000**

** - Highly Significant

TABLE 5: EVALUATION OF MOUTH OPENING IN GROUP A OSMF

GROUP A	No. Of patients	Mean	Standard Deviation	p value
BEFORE	15	25.66	±5.32738	0.000**
AFTER	15	30.20	±6.02613	
DIFFERENCE	15	4.53	±1.18723	0.000**

: ** - Highly Significant

TABLE 6 : EVALUATION OF MOUTH OPENING IN GROUP B OSMF

GROUP B	No. Of patients	Mean	Standard Deviation	p value
BEFORE	15	27.20	±5.69712	0.000**
AFTER	15	29.93	±5.79984	
DIFFERENCE		2.73	±0.70373	0.000**

** - Highly Significant

TABLE 7: COMPARISION OF SUBMUCOSAL THICKNESS - GROUP A

	NO.OF SAMPLES	Mean	Standard deviation	Mean	Standard deviation	p value
RIGHT SIDE	15	0.69	±0.15	0.48	±0.13	0.000**
LEFT SIDE	15	0.69	±0.15	0.48	±0.13	0.000**
BOTH SIDES	30	.7150	±.16604	.4943	±.14628	0.000**

** - Highly Significant

TABLE 8: COMPARISION OF SUBMUCOSAL THICKNESS - GROUP B

	NO.OF SAMPLES	Mean	Standard deviation	Mean	Standard deviation	p value
RIGHT SIDE	15	.74	±0.19	.62	±0.18	0.000**
LEFT SIDE	15	.70	±0.18	.57	±0.17	0.000**
BOTH SIDES	30	.7290	.18830	.5980	.18199	0.000**

** - Highly Significant

TABLE 9: COMPARISION OF ECHOGENICITY GROUP A

		PRE OPERATIVE		POST OPERATIVE		
	No.of Samples	Hyper	Hypo	Hyper	Hypo	P value
RIGHT SIDE	15	11	4	.2	13	0.040*
LEFT SIDE	15	13	2	2	13	0.010*
BOTH SIDE	30	24	6	4	26	0.000**

a - binomial distribution used, * -significant, ****** - highly significant

TABLE 10: COMPARISION OF ECHOGENICITY GROUP B

		PRE OPERATIVE		POST OPERATIVE		
	No.Of Samples	Hyper	Hypo	Hyper	Hypo	p value
RIGHT SIDE	15	12	3	7	8	0.062
LEFT SIDE	15	13	2	4	11	0.012*
BOTH SIDE	30	25	5	11	19	0.001*

[a] binomial distribution used, * -significant, ****** - highly significant

TABLE 11: COREALTION OF MOUTH OPENING WITH SUBMUCOSAL THICKNESS – GROUP A

PEARSON CORELATION		MOUTH OPENING POST OP (p value)	USG PRE OP SUBMUCOSAL THICKNESS (p value)
MOUTH OPENING PRE OP N=15	GROUP A	0.986**(0.00)	- 0.534*(.041)
	GROUP B	0.993** (0.00)	- 0.549*(.034)
USG PRE OP SUBMUCOSAL THICKNESS N=15	GROUP A	- 0.530* (0.042)	0.977**(.00)
	GROUP B	- 0.517*(0. .049)	0.993**(.00)

* -significant, ****** - highly significant

TABLE 12: PERCENTAGE OF IMPROVEMENT IN MOUTH OPENING AND SUBMUCOSAL THICKNESS

%	MOUTH OPENING	SUBMUCOSAL THICKNESS		
		RIGHT	LEFT	OVERALL
GROUP A	17.66%	31.01%	30.72%	30.86%
GROUP B	10.04%	17.09%	18.89%	17.99%

RESULTS AND OBSERVATIONS

A total of 30 patients diagnosed clinically as OSMF were included in the study. The patients were assigned to two groups

- Group A were administered pentoxifylline (oxypentifylline) 400 mg thrice daily for 3 months
- Group B were administered intralesional injection of 0.5ml of LA with 1ml of dexamethasone and 1500 I.U of hyaluronidase biweekly for 6 weeks.

Burning sensation, mouth opening clinically, Submucosal thickness and submucosal echogenicity of buccal mucosa was measured using high frequency ultrasonograpy in both the groups, preoperatively and postoperatively.

A total of 30 patients with age ranging from 23- 54 years, with the mean age being 35.4 years in group A and 32.8 years in group B, were included in the study . The maximum number of subjects was in the age group of 26 – 30 years and minimum were in 46 -55 years of age. Out of 30 subjects enrolled in the study 14 were males and 6 were females. A male predilection was observed **[TABLE 1] [CHART 1 & 2].**

In the present study, stage II is more prevalent than stage III were observed, 19 patients in stage II and 11 patientss in stage III. This is correspond to the study conducted by **Anjum aara et al**[45] who also reported with increased prevalence of stage II compared to stage III **[TABLE 2] [CHART 3 & 4].** Association of stages of OSMF with various variables in the study also analysed **[TABLE 3] [CHART 5 -7].**

In the present study, all the patients in group A showed complete reduction in burning sensation and 2 pstients in group B showed mild persistance of burning sensation after medication. But both the groups showed highly significant reduction

($p<0.001$) in burning sensation **[TABLE 4] [CHART 8]**. A study conducted by **Rajendran et al**, reported statistically significant improvement in burning sensation in 14 patients treated with pentoxifylline for a period of 7 months[43].

In the present study all patients in both groups showed highly significant increase in mouth opening ($p<0.001$), but group A showed marginally higher degree of improvement (mean- 4.53mm) compared to group B (mean – 2.73 mm) **[TABLE 5 & 6] [CHART 9]**. The maximum increase in mouth opening was 7mm and the minimum was 3mm in group A whereas it is 4mm and 2mm respectively in group B.

In the present study, the overall mean pre-operative submucosal thickness of buccal mucosa is about 0.722 mm which range from 0.43mm to 1.12mm. The mean pre operative submucosal thickness of buccal mucosa in group A is about .715 ±.166mm which range from 0.46 mm to 1.12mm, for right side it is about 0.698±.157 mm and for left side is about 0.731±.157mm. However in a study conducted by **Devathambi et al (2013)**[94], mean submucosal thickness of buccal mucosa range from 0.90mm to 2.61mm.The overall mean post operative submucosal thickness of buccal mucosa in group A is about 0.4943±.14628mm which range from 0.26 mm to 0.84mm, for right side is about .482±.134 mm and for left side is about .506±.161mm, both are found to be highly significant **[TABLE 7] [CHART 10].** This is the first study which evaluates the post operative submucosal thickness of buccal mucosa after medication.

In case of group B , mean submucosal thickness is about .729±.188mm which range from 0.43 mm to 1.08mm, whereas in right side is about .748±.197mm and for left side is about .709±.183mm. The overall improvement is about .598±..181mm which range from 0.43 mm to 1.08mm, whereas in right side is about

.6207±.18786mm and for left side is about .5753±.17948, which also showed highly significant value **[TABLE 8] [CHART 11].**

In the present study, McNmer test is used to evaluate the echogenicity because of binomial distribution of values. Pentoxifylline group showed significant resulst in echogenicity on both right and left side ($p<0.005$) individuallyand combined together showed highly significant result ($p<0.001$) **[TABLE 9] [CHART 13&14].**

Dexamethasone group also showed similar significant result individually and highly significant result on combined together of both sides **[TABLE 10].** This is the first study which evaluates echogenicity of submucosa on OSMF patients to analyse the outcome of different medical mode of treatment.

Comparision of improvement in mouth opening and submucosal thickness after treatment was evaluated on both groups using Pearson correlation. Both showed highly significant result($p<0.001$) on both groups . Cross comparison of mouth opening with submucosal thickness was also done on both groups, which showed significant result ($p<0.05$) on both the groups **[TABLE 11] [CHART 15].**This implies that both medical mode of treatment are effective in improving clinical symptoms.

This present study also concluded that the group A showed **17.66%** improvement in mouth opening and **30.86%** improvement in submucosal thickness compared to **10.04%** and **17.99%** improvement in group B patients respectively **[TABLE 12] [CHART 16].** This data clearly signifies that both the pentoxifylline and intralesional injection of dexamethasone with hyaluronidase are effective in the management of OSMF patients, but pentoxifylline have little edge over it.

CHART 1

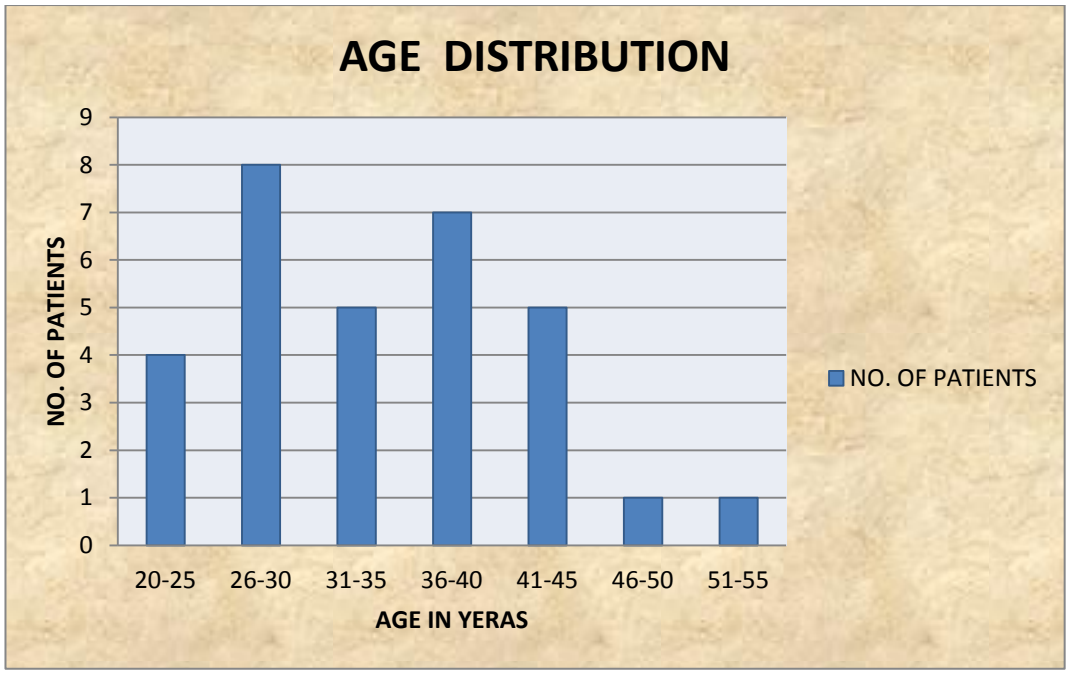


CHART 2

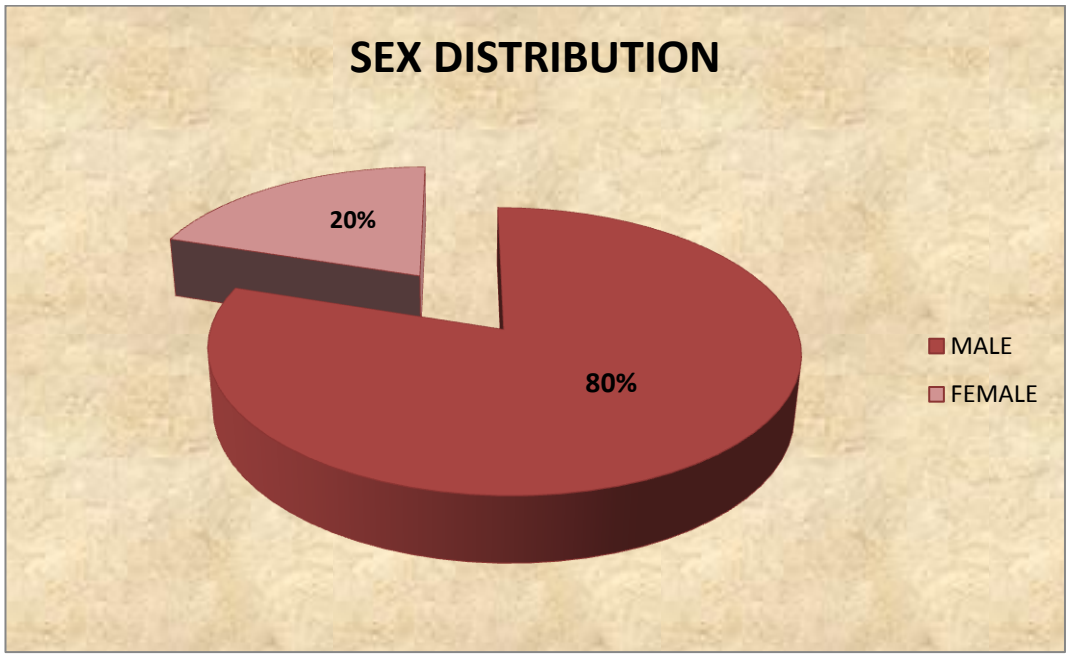


CHART 3

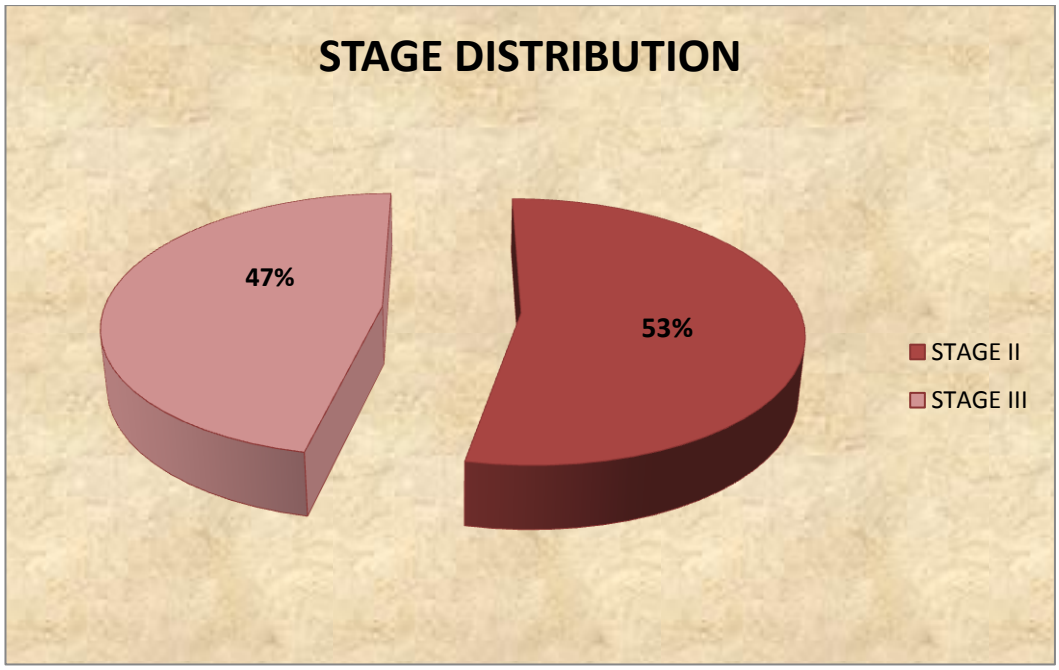


CHART 4

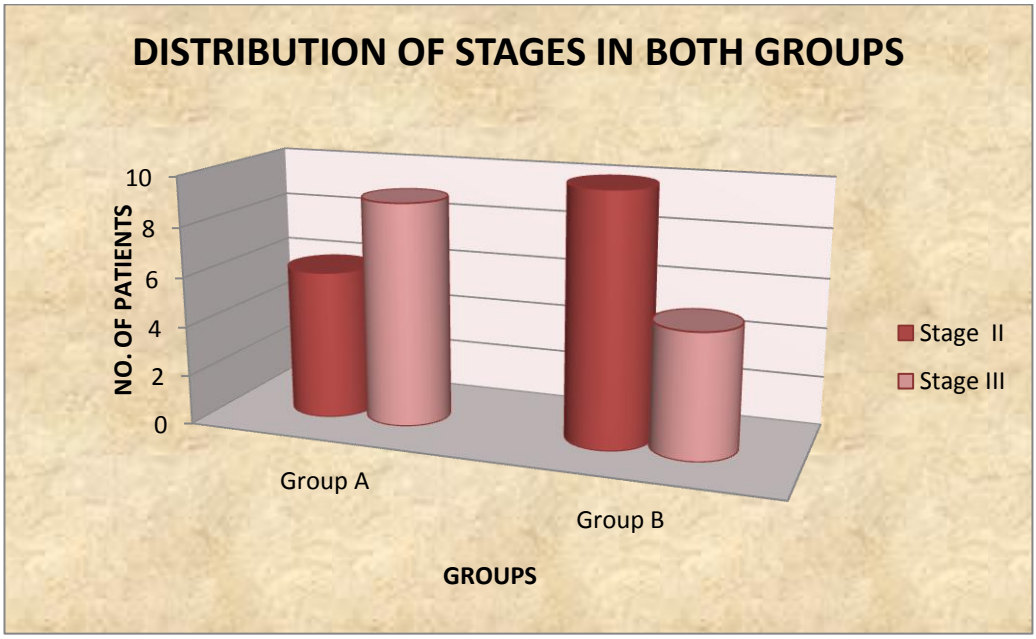


CHART 5

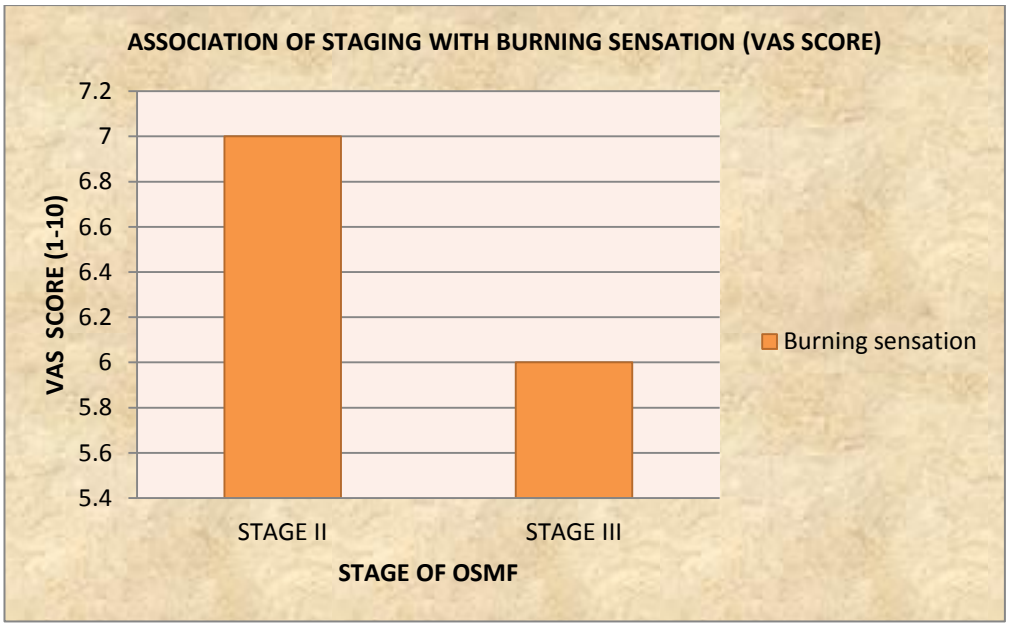


CHART 6

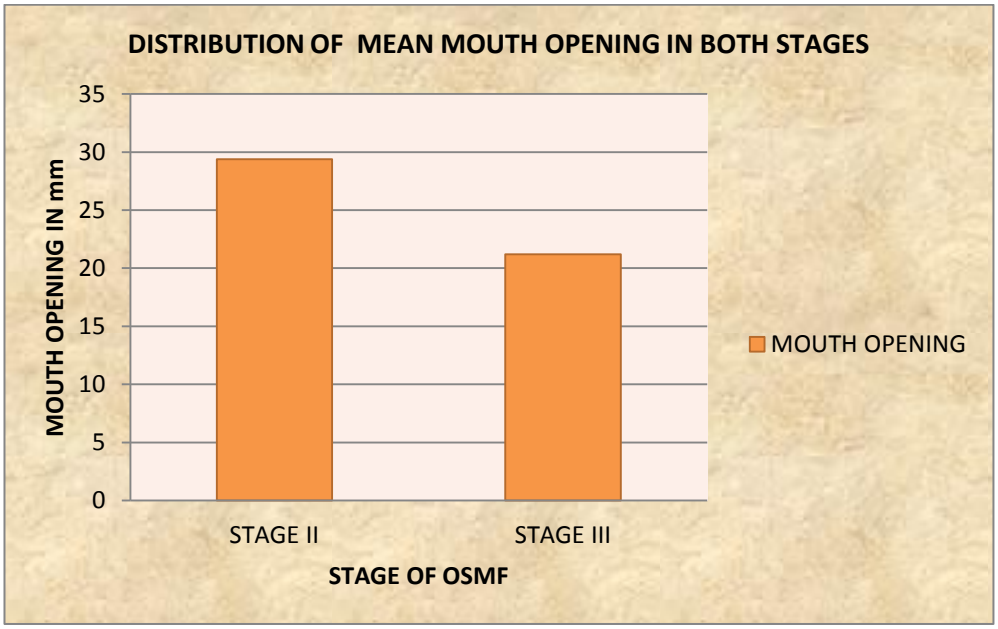


CHART 7

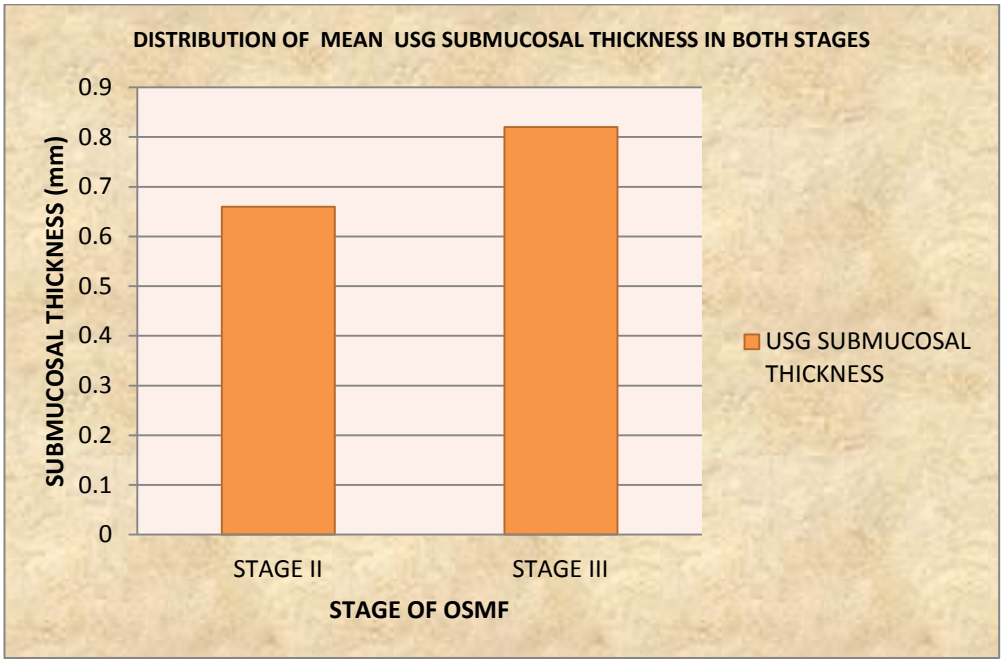


CHART 8

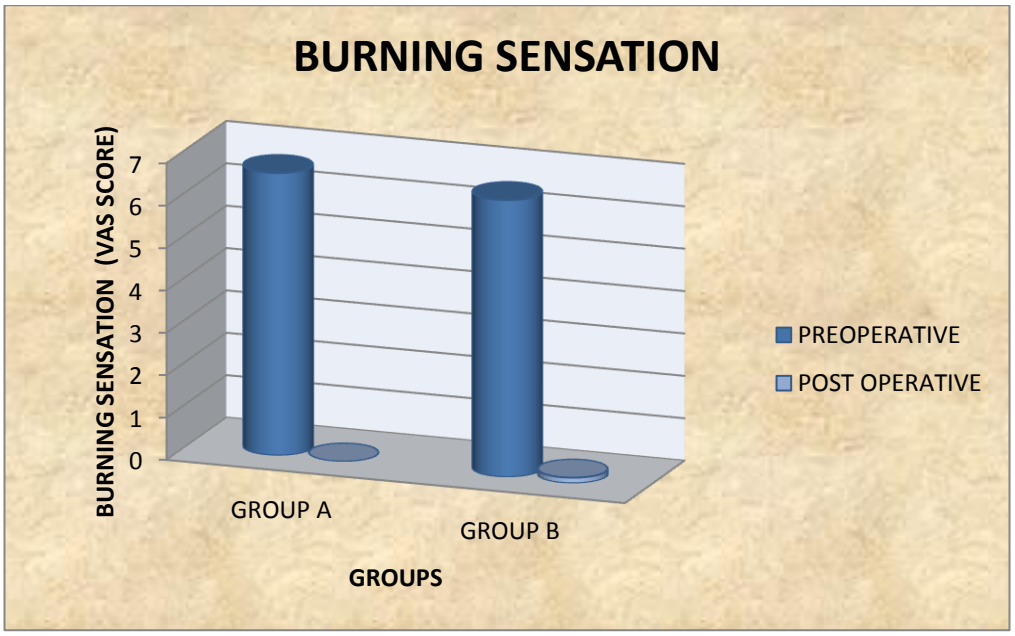


CHART 9

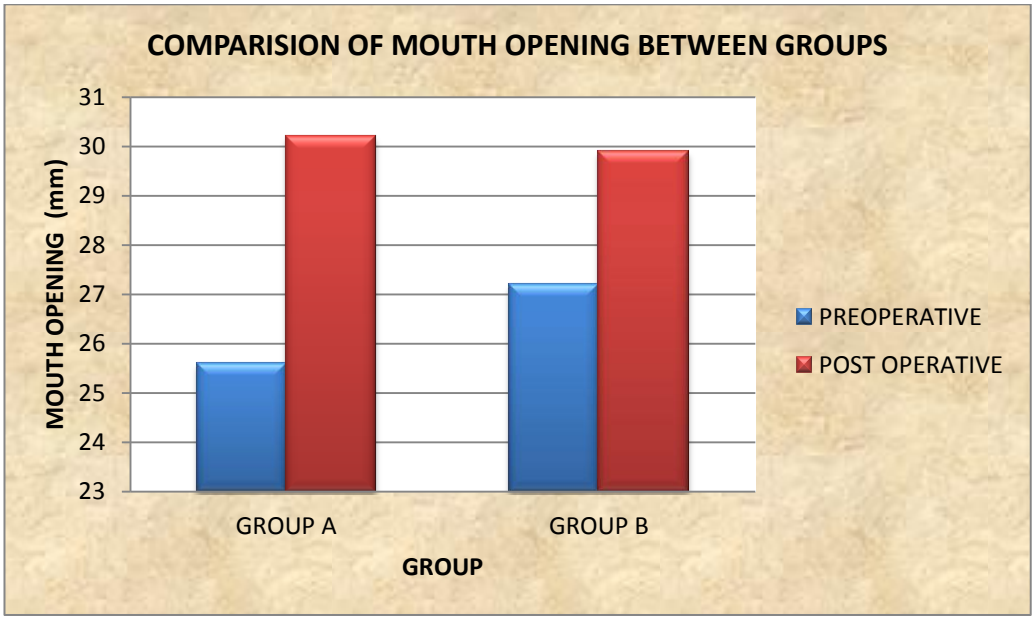


CHART 10

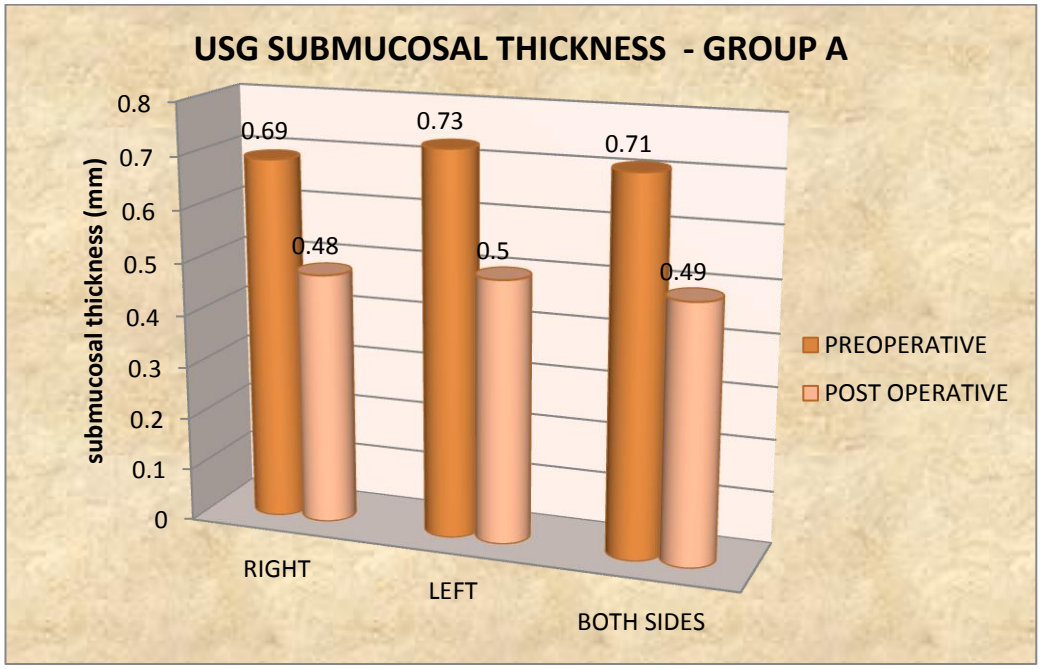


CHART 11

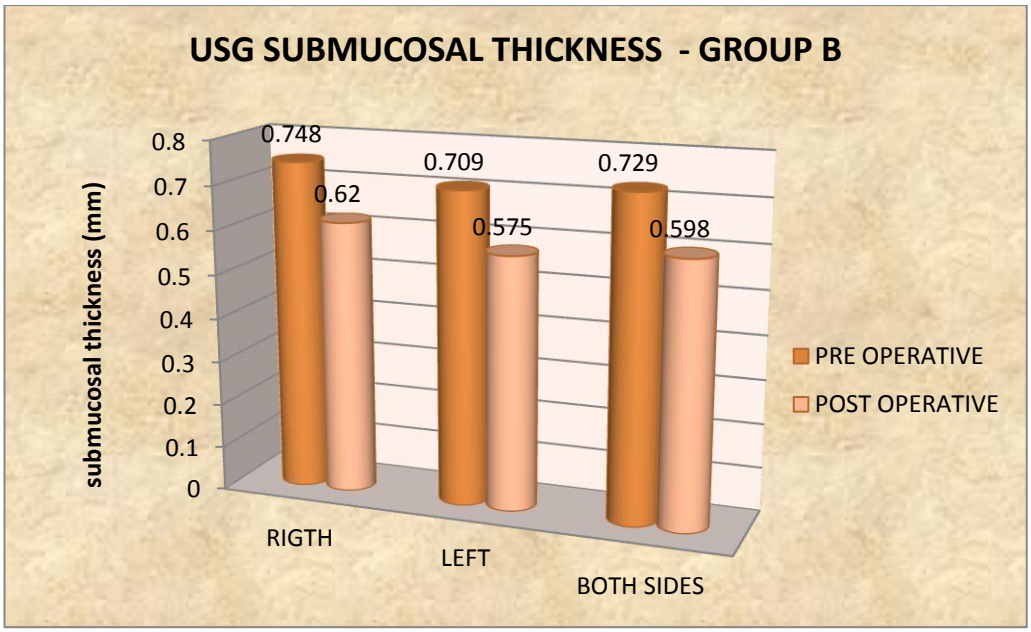


CHART 12

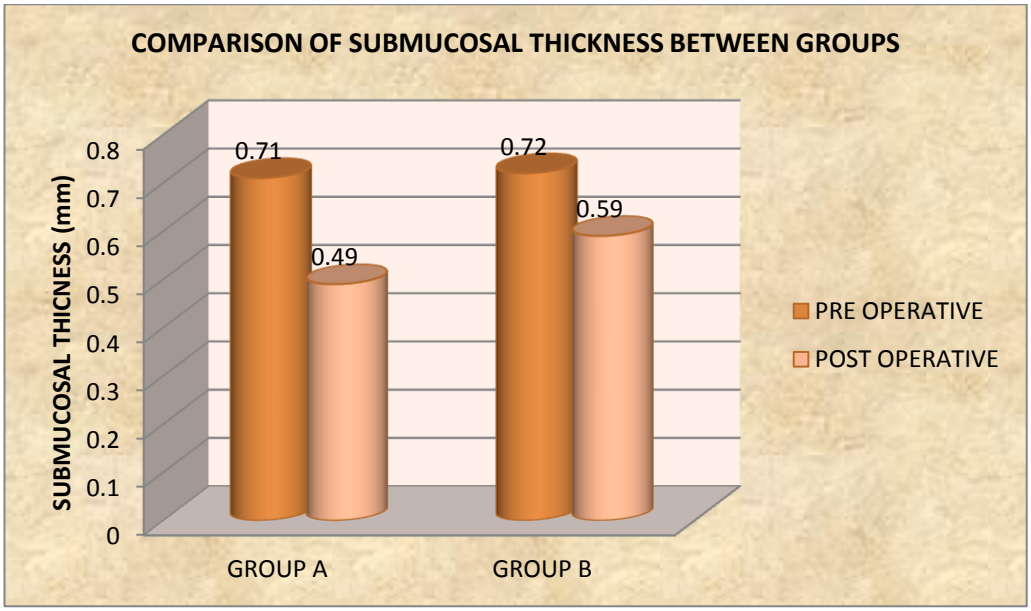


CHART 13

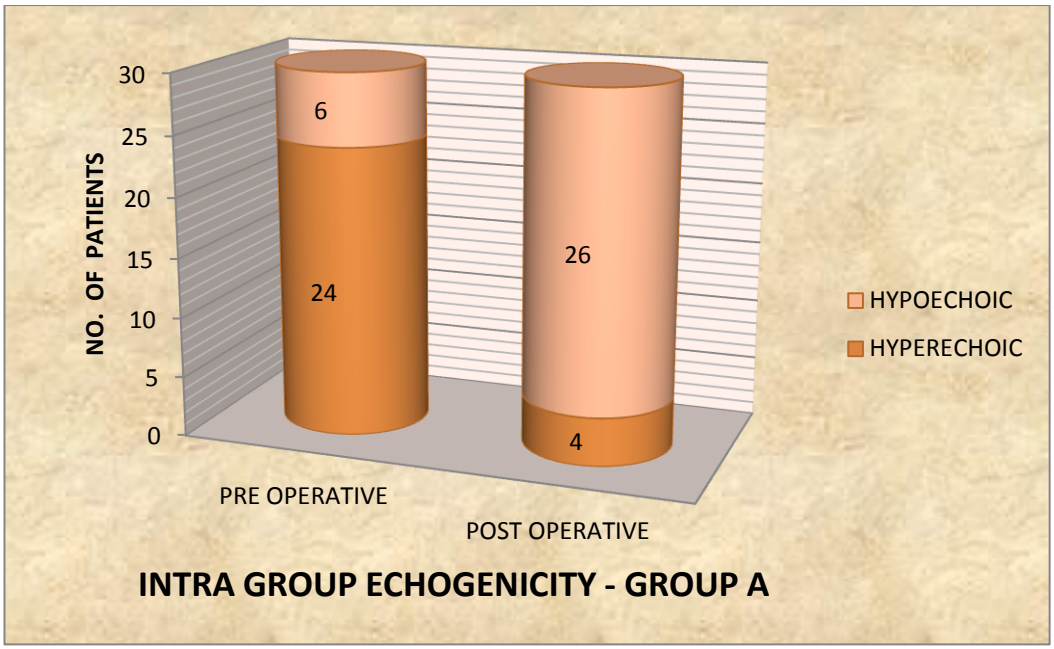


CHART 14

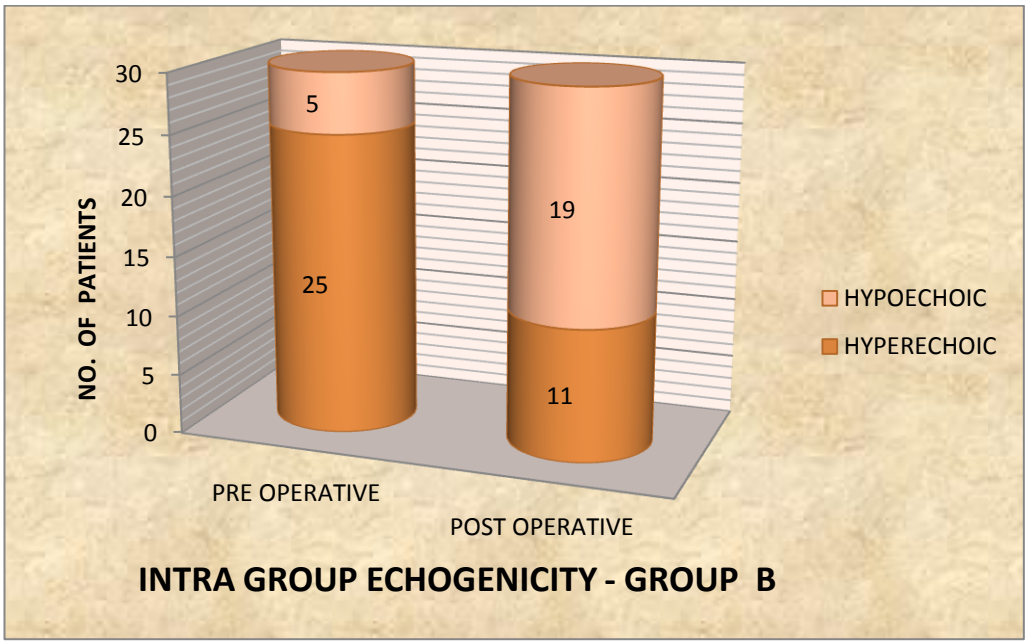


CHART 15

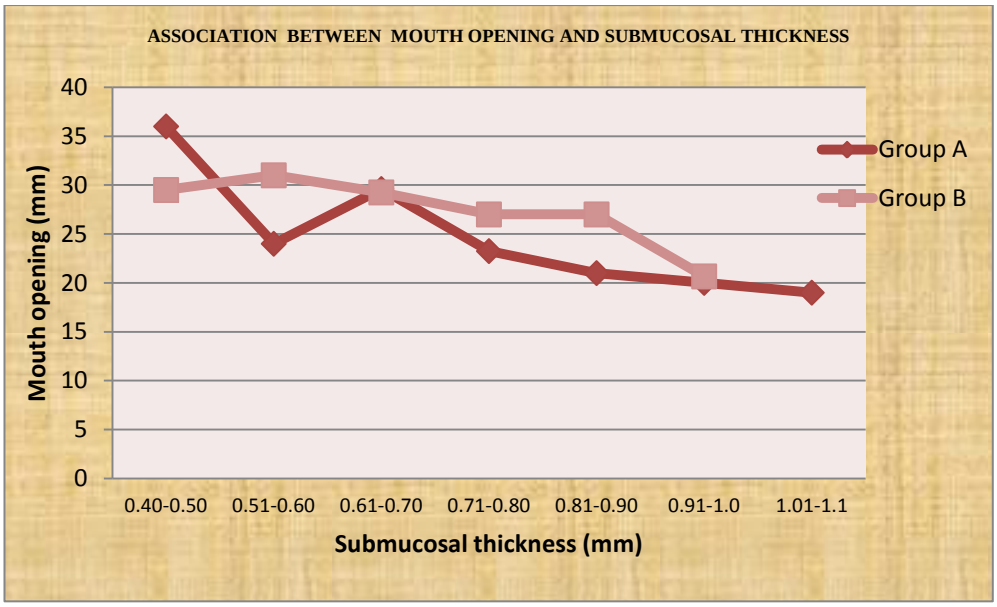
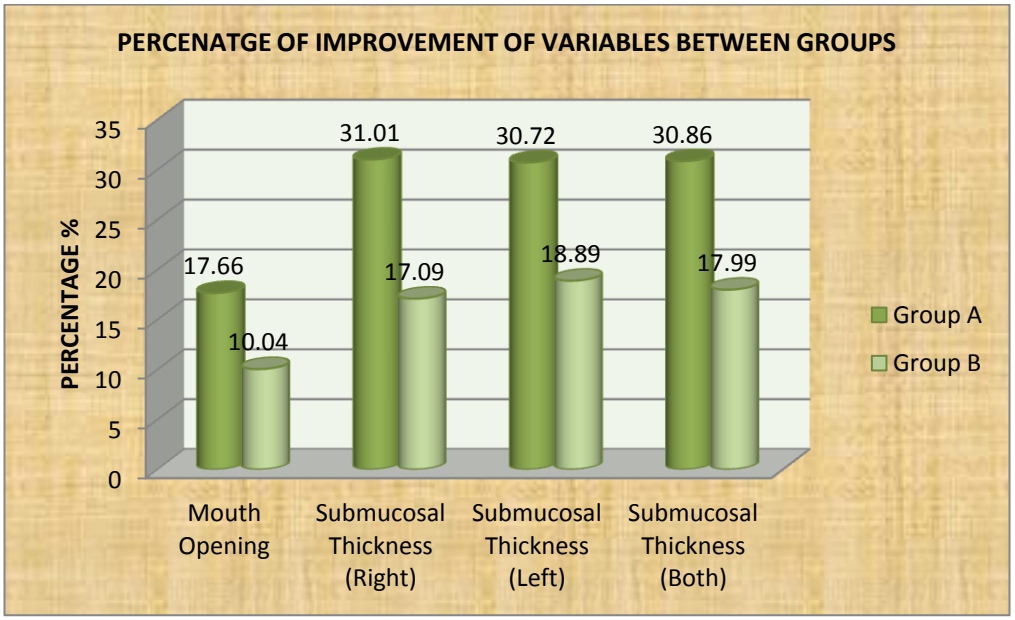


CHART 16



DISCUSSION

Oral submucous fibrosis is a chronic debilitating pre-malignant condition affecting millions of individuals worldwide and most commonly seen in the Asian subcontinent. The prevalence in India ranges from 0.2%-1.2%[5] and malignant transformation rate of 3-7.6%[95]. Despite extensive research, the etiology of OSMF still remains largely unknown and the pathogenesis is yet to be completely elucidated. Several factors such as chili consumption, areca nut chewing[7], nutritional deficiencies[96], genetic susceptibility[28], autoimmunity and collagen disorder have been suggested to be involved in the pathogenesis of this condition. Based on clinical, epidemiological and in vitro studies, areca nut chewing is considered as the important predisposing factor.

Various modalities were proposed for the treatment of OSMF. Medical treatments were designed to increase the mouth opening and improve other signs and symptoms associated with this disease. The different categories of drugs used include steroids, antioxidants, immunised cow milk[9], colchicine[10], vitamins, microelements, enzymes, vasodilators and immunomodulators.

The present drug treatments in general are empirical at best and a definitive cure for the disease still remains elusive. Currently intralesional steroid with or without hyaluronidase is considered to be the effective medical management for OSMF. But because of the painful injection, need of attention towards frequent visits, dexterous skill for submucosal injection warrant the intralesional therapy quite unsatisfactory.

Pentoxifylline, a methylxanthine derivative with potent hemorrheologic properties, was therefore considered to be effective in treating OSMF. The anti-

inflammatory and immunomodulatory actions of pentoxifylline seems to have definite therapeutic advantage in the management of OSMF. As OSMF is a chronic mucosal inflammatory disease, control of the inflammation or the factors influencing the inflammatory process should form the basis of definitive management. Pentoxifylline has the ability to decrease the production of tumour necrosis factor alpha (TNF-α), an important mediator of the inflammatory response.The property of pentoxifylline that may be far reaching in the management of OSMF is possibly its inhibitory effect on fibroblast proliferation and fibrinolysis.

Most of the changes associated with OSMF are an irreversible process, treatment outcomes are difficult to evaluate precisely, as those alterations are occurring at submucosal level. Hence evaluation of clinical symptoms may not precise to evaluate the efficacy of medical therapy. In order to evaluate this condition better, monitoring the improvement at submucosal level is mandatory. Currently an innovative imaging modality, USG provides visualisation of changes at submucosal level at real time basis.

USG imaging is considered to be a "real- time" imaging, wherein the reflected echoes are processed at a rapid frame rate to allow simultaneous perception of physiologic motion. The echogenecity of a tissue primarily relates to its 'stiffness', the chief source of which is collagen, the content and arrangement of collagen within tissue. It also provide both qualitative and quantitative assessment of mucosal tissue.

Thus the present study is indent to evaluate and compare the effectiveness of oral pentoxifylline with respect to combined intralesional injection of dexamethasone and hyaluronidase in OSMF patients using USG by measuring the changes in submucosal thickness and echogenisity before and after treatment in both the groups.

To best of our knowledge, this is the first study based on USG to evaluate the efficacy of pentoxifylline therapy in OSMF patients.

In our study we administered 400mg of pentoxifylline thrice daily for a period of three months for patients in group A and 1ml of dexamethasone with 1500 I.U of hyaluronidase in 0.5ml of local anesthesia biweekly for 6 weeks in group B patients. The recommended adult dosage of oral pentoxifylline is 400mg three times a day with meals for hemorrheologic indications, which is also recommended for most other clinical applications[80]. Only few clinical trials regarding the effectiveness of pentoxifylline in the management of OSMF, has been reported till date. The study conducted by **Anjum aara et al** used a dosage of 400mg of pentoxifylline two times to three times a day for a period of 3 months[45]. The outcome of this study showed significant clinical improvement with minimal side effects. Therefore we decided to use thrice daily regimen for three months.

A total of 30 patients with age ranging from 23- 54 years, with the mean age being 35.4 years in group A and 32.8 years in group B, were included in the study . The age distribution in this study is correlated with that stated by other authors[97]. Out of 30 subjects enrolled in the study 24 were males and 6 were females. A male predilection was observed. All the 30 patients in the study had a pan chewing habit. In another study from Chennai, out of 185 patients, only one patient did not have a history of areca nut chewing[19].

The symptoms commonly reported include burning sensation of mouth, reduced mouth opening, decreased salivation and altered taste perception. **Burning sensation** may be attributed to an atrophic epithelium, but currently the morphologic evidence of increased cell death was failed to be noticed in OSMF epithelium when compared to site adjacent healthy oral mucosa[68]. Stomatitis resulting from nutritional

deficiency may also lead to burning sensation. Several investigators have reported nutritional deficiencies such as anemia, vitamin, iron, and protein deficiencies in patients with OSMF[7]. The burning sensation may also be aggravated by the xerostomia that is seen in the later stages of the disease.

Burning sensation of the mouth and a reduction in mouth opening was present in all the 30 patients enrolled in the study. There was a complete reduction in the burning sensation in all the 15 patients in group A and 13 patients in group B. Two of the patient in group B reported with mild persistence of burning sensation even at the end of the treatment. Both the groups showed highly significant ($p<0.001$) reduction in burning sensation. The improvement in regional blood flow by pentoxifylline could explain the significant reduction in burning sensation observed in our study. Treatment of OSMF with intralesional injections of corticosteroids (dexamethasone or triamcinolone acetonide), hyaluronidase and chymotrypsin has been reported to reduce burning sensation in 82% - 94% of OSMF patients[42]. All the patients in our study treated with pentoxifylline showed highly significant reduction in burning sensation.

Restricted mouth opening is the most debilitating feature associated with OSMF. As the disease progresses, thick fibrous bands appear in the submucosal layer of the oral soft tissues. This progressive oral fibrosis is usually bilateral and causes an increasing restriction of mouth opening. The mouth opening ranged from 16-36mm was observed in our study. In a study conducted in Chennai, majority (76%) of the patients had a mouth opening between 20-45mm. All the 30 patients in the study showed significant increase in the degree of mouth opening. However pentoxifylline showed slightly higher percentage than (17.96%) than dexamethasone group (10.04%).

No known treatment for OSMF can restore the mouth opening to normal, although some medical and surgical interventions may result in improvement. Significant alleviation in mouth opening by pentoxifylline might be due to combined physiological action of anti-inflammatory, fibrinolytic, immunomodulating and rheologic modifying property of the drug altogether.

Ultrasonogarphic evaluation of submucosal thickness and echogenicity of buccal mucosa reflect degree of deposition of collagen fibre and stiffness of mucosa. Hence measuring this parameter will be helpful in screening, assessing the extension of the fibrosis and the effectiveness of treatment outcome. And also noted that 30% improvement in submucoal thickness had been noticed in group A and only 17 % improvement was noticed in group B. similarly mouth opening also showed marginally better improvement in group A (17.96 %) and group B (10.04%). Eventhough, both the groups are highly significant, group A showed marginally better changes in all parameters compared to group B.

In study conducted by **Anjum Aara et al**[45]**,** dexamethasone group showed better result compared to pentoxifylline group. But in their study, all parameters were of clinical like burning sensation, mouth opening and cheek flexibility. And also they prescribed twice daily dose of pentoxifylline for the first four week, which may have influenced the result of their study.

ADVERSE EFFECTS:

Most of the side effects caused by pentoxifylline involve the gastrointestinal system and central nervous system. In our study pentoxifylline was generally well tolerated by almost all of the patients. Mild gastric irritation was experienced by two patient and Giddiniess in another patient but neither of the patients had reason to

discontinue the therapy. Patient with gastric irritation was managed by advised to take the medication with meals. In the other reported study where Pentoxifylline has been used in 15 OSMF, one patient experienced continued gastric irritation warranting cessation of therapy. No other reportable side effects or complications were recorded from any of the other patients in the same study.

LIMITATION OF THE STUDY:

The present study was conducted on 30 patients, 15 in each group. A larger sample size would have allowed for better observation and analysis of the disease findings and the effects of the intervention. Duration of pan chewing with severity of OSMF was not considered, which may be helpful in assessing the implication of drug in treatment outcome. The study parameters were recorded only during the duration of the treatment, which were 3 months in group A and 6 weeks in group B. There was no follow up after cessation of active medication. Post-treatment follow up would have allowed for documentation of sustained effect of the intervention and possible relapse.

. USG evaluation was done only before and after the end of the treatment, during the course of treatment was not performed. It may be helpful in evaluating initial appearance of improvement after medication. And also dexterous skill and experience is required for manipulation of ultrasonography and values may change from observer to observer as there is no definitive landmark to define. Hence we took multiples values and average of which is tabulated and analysed. We used extraoral transducer for USG evaluation because of non availability of intraoral transducer, which may be helpful in further detailed analysis of oral submucosa including vascularity.

SUMMARY AND CONCLUSION

Oral submucous fibrosis is a common pre-malignant condition affecting the oral mucosa with more prevalence among Indian population. Various treatment modalities have been elucidated in order to alleviate the symptoms associated with OSMF. Currently oral pentoxifylline has been proved to have beneficial result in treating OSMF because of its anti-inflammatory, fibrinolytic ,immunomodulatory and rheologic modifying property. The present study was done to evaluate the effectiveness of oral pentoxifylline in the management of OSMF and the efficacy of USG in evaluating that effectiveness, as it provides both qualitative and quantitative assessment of oral mucosa.

Several categories of drugs have been used in the treatment of OSMF but their effectiveness leaves much to be desired and no treatment regimen has afforded definitive cure. While Oral administration limits the concentration of drugs in lesional tissue and increases the potential for side effects, the Intralesional injections are associated with significant mechanical injury and non compliance on the patient's part because of the accompanying discomfort and pain.

In this study both pentoxifylline and dexamethasone group showed significant improvement in mouth opening, reduction in burning sensation. Pentoxifylline appears to be well tolerated. Only 2 patients experienced side effects, but neither had reason to discontinue the therapy. Hence pentoxifylline can be a good alternative in the management of OSMF for whom intralesional steroids or hyaluronidase are contraindicated, for those who cannot make frequent visit and to avoid pain due to injection. And most importantly pentoxifylline is cost effective and more compliant to the patient.

Furthermore, qualitative and quantitative assessment of pentoxifylline effectiveness in treating OSMF was assessed by USG showed marked changes in submucosal thickness and echogenicity in lieu with the clinical improvement. Hence USG can be considered as a valuable tool in assessing the severity, extension, disease progression and treatment outcome objectively and efficiently. But further descriptive study is required to substantiate the sensitivity of USG in OSMF evaluation, in all respects.

However the present study involved a small sample size, thus the results of our study need to be confirmed in a larger population of OSMF patients with a longer period of follow up. Also further study is required to assess the effectiveness of pentoxifylline on the basis of extent of efficacy in different age groups, at various stages of OSMF, and duration of habit associated with OSMF.

The future directions in the management of OSMF should thus include development of treatment regimens that combine different drugs or uses sequential therapy. Though the individual treatment response to pentoxifylline showed significant improvement, additional studies are required to further establish the role of pentoxifylline in observed therapeutic effects. Quality randomized, controlled trials and increasing the global awareness of the disease for greater inflow of research data and research on possible treatment approaches is the need of the hour. Lastly, OSMF is a preventable disease; simple public health awareness of the harmful effects of chewing areca and other products could go a long way in combating this debilitating disease.

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INSTITUTIONAL ETHICAL COMMITTEE

Tamil Nadu Government Dental College and Hospital, Chennai - 3
Telephone No. 044 2534 0343
Fax 044 2530 0681

Ref.No.0430/ DE/ 2010 Date: 21.02.2014

Title of the work: "Ultrasonographic evaluation of Oral submucous fibrosis treated with Oral Pentoxifylline and intralesional Dexamethasone with Hyaluronidase"

Principal investigator: **Dr.M.Suresh Kumar,**
II Year MDS

Department : Oral Medicine and Radiology,
Tamil Nadu Government Dental College and Hospital, Chennai - 3

The request for an approval from the Institutional Ethical Committee (IEC) considered on the IEC meeting held on **29.01.2014** at the Principal's Chambers Tamil Nadu Government Dental College and Hospital, Chennai – 3

"Advised to proceed with the study"

The Members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above , submitted by the principal investigator.

The principal investigator and their team are directed to adhere the guidelines given below:

1 .You should get detailed informed consent from the patients / participants and maintain confidentiality

2. you should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.

3 You should inform the IEC in case of any change of study procedure , site and investigation or guide.

4. You should not deviate from the area of work for which you have applied for ethical clearance

5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the institution (s)

6. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.

7 .You should submit the summary of the work to the ethical committee on completion of the work.

8. You should not claim funds from the Institution while doing the work or on completion.

9.You should understand that the members of IEC have the right to monitor the work with prior intimation

10. Your work should be carried out under the direct supervision of your Guide / Professor.

SECRETARY

CHAIRMAN

INFORMED CONSENT FORM

STUDY TITLE:

ULTRASONOGRAPHIC EVALUATION OF ORAL SUBMUCOUS FIBROSIS TREATED WITH ORAL PENTOXIFYLLINE AND INTRALESIONAL DEXAMETHASONE WITH HYALURONIDASE

Name: Age / Sex: O.P.No:

Address: Serial No:

Tel. no:

I, _____age____years

Exercising my free power of choice, hereby give my consent to be included as a participant in the study "**Ultrasonographic Evaluation of Oral Submucous Fibrosis treated with Pentoxifylline and Intralesional Dexamethasone with Hyaluronidase**".

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking and other systemic illness that I have.
- I agree to report to the doctor for a regular follow-up as and when required for the research.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Signature / Thumb impression

Name of the investigator Signature

Date

INFORMATION SHEET

- We are conducting **an Ultrasonographic Evaluation of Oral Submucous Fibrosis treated with Oral Pentoxifylline and Intralesional Dexamethasone with Hyaluronidase**" among patients attending TNGDCH, Chennai and for that study we are selecting patients.
- The purpose of the study is to evaluate the therapeutic benefit of oral Pentoxifylline in oral submucous fibrosis patients using ultrasonography..
- The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the investigator Name of the patient

Signature of investigator Signature of patient

Date:

ஆராய்ச்சி தகவல் தாள்

வாய் இறுக்கு நோய்க்கு பென்டாக்ஷிபிலின் மாத்திரை மற்றும் டெக்ஸாமெதசோன் ஹயலூரோனிடேஸ் இடைத்திசு சிகிச்சையின் ஆற்றலை மீயொலி கொண்டு செய்யும் பகுத்தாய்வு குறித்து ஆராய்ச்சி செய்யும் பொருட்டு தமிழ்நாடு அரசு பல் மருத்துவக் கல்லூரி மற்றும் மருத்துவமனைக்கு வரும் நோயாளிகளை நாங்கள் தேர்வு செய்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்கும் நோயாளிகளின் விபரங்கள் ஆய்வு முடிவும் வரை இரகசியமாக வைக்கப்படும். ஆராய்ச்சியின் முடிவு பற்றிய பாதிப்புகள் அல்லது வெளியீடுகள் யாருடைய தனிப்பட்ட விவரங்களும் பகிர்ந்து கொள்ளப்படமாட்டாது.

இந்த ஆராய்ச்சியில் பங்கேற்கும் உங்கள் முடிவு தன்னிச்சையானது. இந்த ஆராய்ச்சியில் பங்கேற்கும் நீங்கள் எந்தநேரத்திலும் விலகிக் கொள்வதற்கும் உங்களுக்கு வாய்ப்பு உள்ளது. உங்கள்ின் இந்த தீர்மானத்தினால் உங்களுக்கு இம்மருத்துவமனையில் வழங்கப்படும் பயன்களில் எவ்வித மாற்றமும் இருக்காது.

இந்த சிறப்பு ஆய்வின் முடிவுகள், இந்த ஆய்வின் முடிவில் அல்லது ஆய்வின்போது ஏற்படும் எதிர்மறையான விளைவுகளை அந்நோயாளிகளின் நலன்கருதியோ அல்லது சிகிச்சையளிக்கும் பொருட்டோ நோயாளிக்கு தெரிவிக்கப்படும்.

ஆய்வாளரின் கையொப்பம் **பங்கேற்பாளர் கையொப்பம்**

தேதி :
இடம் :

சுய ஒப்புதல் படிவம்

<u>ஆய்வு செய்யப்படும் தலைப்பு</u>

வாய் இறுக்கு நோய்க்கு பென்டாக்ஷிபிலின் மாத்திரை மற்றும் டெக்ஸாமெதசோன் ஹயலூரோனிடேஸ் இடைத்திசு சிகிச்சையின் ஆற்றலை மீயொலி கொண்டு செய்யும் பகுத்தாய்வு குறித்து ஆராய்ச்சி

ஆராய்ச்சி நிலையம் : அரசு பல் மருத்துவக் கல்லூரி
சென்னை - 600 003

பங்கு பெறுபவரின் பெயர் :

பங்கு பெறுபவரின் எண் :

பங்கு பெறுவரின் பிறந்த தேதி : _____ / _____ /_____
தேதி மாதம் வருடம்

இந்த ஆராய்ச்சி சம்பந்தமாக நான் மேலே கூறப்பட்ட தகவல் படிவத்தை முழுமையாக படித்துப் பார்த்தேன் என்று உறுதி கூறுகிறேன்.

நான் இது தொடர்பான அனைத்து கேள்விகளுக்கும் நிறைவான பதில்கள் பெறப்பட்டேன்.

இந்த ஆய்வின் எனது பங்கு தன்னிச்சையானது என்றும் எந்த நேரத்திலும் இந்த ஆய்வில் இருந்து சட்ட உரிமைகள் பாதிக்கப்படாமல் விலகிக் கொள்ள சம்மதிக்கிறேன்.

மருத்துவ ஆய்வு அதிகாரிகள், எனது சிகிச்சை தொடர்பான பதிவேடுகளை பார்வையிடவும் எந்த நேரத்திலும், ஆய்வில் இருந்து நான் விலகினாலும் பார்வையிட சம்மதிக்கிறேன். எனது அடையாள குறிப்புகள் மூன்றாவது நபருக்கு தெரிவிக்கப்படமாட்டாது என்று புரிந்து கொண்டேன்.

இந்த ஆய்வு அறிக்கைகளை பயன்படுத்தவும், வெளியிடவும், நான் சம்மதிக்கிறேன். ஆய்வாளர் எனது மருத்துவக் குறிப்புகளை வெளியிட தடையாக இருக்கமாட்டேன் என உண்மையாக சம்மதிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம் ..... இடம்..... தேதி.....

கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் .....

ஆய்வாளரின் கையொப்பம் ..... இடம்..... தேதி.....

ஆய்வாளரின் பெயர் .....

DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

TAMIL NADU GOVT. DENTAL COLLEGE & HOSPITAL, CHENNAI -3

CASE PROFOMA

ULTRASONOGRAPHIC EVALUATION OF ORAL SUBMUCOUS FIBROSIS TREATED WITH ORAL PENTOXIFYLLINE AND INTRALESIONAL DEXAMETHASONE WITH HYALURONIDASE

Date: Serial no:

Name: O.P No:

Age/Sex:

Address:

Phone no:

Occupation: Income:

Centre : 1. Department Of oral medicine and radiology,

Tamil Nadu Govt Dental College & Hospital, Chennai -3

2. Bernard Institute of Radiology(BIR),

Rajiv Gandhi Government General Hospital , chennai

Presenting complaint with duration:

Past medical history:

Past dental history:

Personal history:

Marital status:

Family history:

A) Smoking habit:
   - Material used:
   - Frequency :
   - Duration of the habit:

B) Chewing habit:
   - Material used:
   - Frequency :
   - Duration of the habit:

C) Other habits (alcohol, snuff):

CLINICAL EXAMINATION

Extraoral Examination:

Intraoral examination:

- Mouth opening (Inter incisal distance):
- Labial and buccal mucosa:
- Hard and soft palate, Uvula:
- Tongue
- Floor of the mouth:
- Retromolar trigone:

Clinical diagnosis: (with grading)

Investigations:

1) Laboratory investigations:

   A) Blood:
      Total WBC count: Erythrocyte sedimentation rate:
      Differential count: Bleeding time:
      Haemoglobin %: Clotting time:
      Peripheral smear:

   B) Urine:
      Glucose:
      Albumin:

OTHERS INVESTIGATION:

Final diagnosis:

Treatment plan:

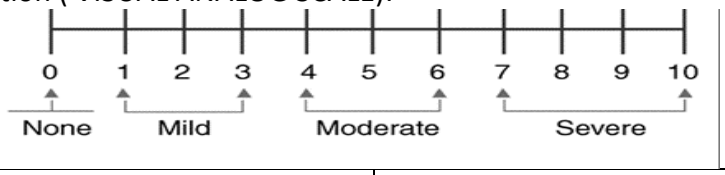
CLINICAL AND ULTRASONOGRAPHIC ASSESMENT FORM

NAME: AGE /SEX: OP. NO: Serial NO. :

DIAGNOSIS : GFROUP ASSIGNED:

CLINICAL PARAMETERS:

Burning Sensation (VISUAL ANALOG SCALE):



BEFORE TREATMENT (VAS score)	AFTER TREATMENT(VAS score)

Mouth Opening : (Interincisal Distance)

BEFORE TREATMENT		AFTER TREATMENT	
RIGHT	LEFT	RIGHT	LEFT
…….mm	…….mm	…….mm	…….mm

USG EVALUATION :

Submucosal Thickness

BEFORE TREATMENT (mm)		AFTER TREATMENT(mm)	
RIGHT	LEFT	RIGHT	LEFT

Echogenicity: (hyperechoic/hypoechoic) score 1/2

BEFORE TREATMENT(score)		AFTER TREATMENT(score)	
RIGHT	LEFT	RIGHT	LEFT

SIGANATURE OF PG STUDENT SIGNATURE OF GUIDE

DATE :

TRIPARTITE AGREEMENT

This agreement hereinafter the "Agreement" is entered into on this day……. between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai- 600 003, (hereinafter referred to as, 'the college')

And

Dr. S. JAYACHANDRAN, M.D.S., Ph.D., aged 50 years working as **Professor** in Department of Oral medicine and Radiology at the college, having residence address at A.M -16, TNHB quarters, tod hunter nagar, saidapet,Chennai – 15.(herein after referred to as the 'Principal Investigator')

And

Dr. SURESH KUMAR M, aged 30 years currently studying as final year **Postgraduate student** in the Department of Oral Medicine and Radiology, Tamil Nadu Government Dental College and Hospital, Chennai -3 (hereafter referred to as the 'PG and co- investigator') residing at no.166, Kottai Allah Kovil Street, Packiyam Nagar, Patukkottai-614601

Whereas the 'PG student as part of her curriculum undertakes to research on "**Ultrasonographic Evaluation of Oral Submucous Fibrosis treated with Oral Pentoxifylline and Intralesional Dexamethasone with Hyaluronidase**" for which purpose the Principal investigator shall act as Principal investigator and the College shall provide the requisite infrastructure based on availability and also provide facility to the PG student as to the extent possible as a Co-investigator

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard

Now this agreement witnessed as follows :

1. The parties agree that all the Research material and ownership therein shall become the vested right of the college, including in particular all the copyright in the literature including the study, research and all other related papers.
2. To the extent that the college has legal right to do go, shall grant to licence or assign the copyright so vested with it for medical and/or commercial usage of interested persons/entities subject to a reasonable terms/conditions including royalty as deemed by the college.
3. The Royalty so received by the college shall be shared equally by all the three parties.

4. The PG/Research student and PG/Principal Investigator shall under no circumstances deal with the copyright, Confidential information and know-how-generated during the course of research/study in any manner whatsoever, while shall sole west with the college.
5. The PG student and Principal Investigator undertake not to divulge (or) cause to be divulged any of the confidential information or, know-how to anyone in any manner whatsoever and for any purpose without the express written consent of the college.
6. All expenses pertaining to the research shall be decided upon by the principal investigator/Co-investigator or borne sole by the PG student.(co-investigator)
7. The college shall provide all infrastructure and access facilities within and in other institutes to the extent possible. This includes patient interactions, introductory letters, recommendation letters and such other acts required in this regard.
8. The Principal Investigator shall suitably guide the Student Research right from selection of the Research Topic and Area till its completion. However the selection and conduct of research, topic and area research by the Student Researcher under guidance from the Principal Investigator shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for this purpose.
9. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the PG student, under guidance from the Principal Investigator, the decision of the College shall be binding and final.
10. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.

In witness where of the parties herein above mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its **Principal** **PG Student**

Witnesses **Student Guide**

1.

2.